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NEWS	2		"Ask CAS" for self-help around the clock
NEWS	3	SEP 09	CA/CAplus records now contain indexing from 1907 to the present
NEWS	4	AUG 05	New pricing for EUROPATFULL and PCTFULL effective August 1, 2003
NEWS	5	AUG 13	Field Availability (/FA) field enhanced in BEILSTEIN
NEWS	6	AUG 18	Data available for download as a PDF in RDISCLOSURE
NEWS	7	AUG 18	Simultaneous left and right truncation added to PASCAL
NEWS	8	AUG 18	FROSTI and KOSMET enhanced with Simultaneous Left and Righ Truncation
NEWS	9	AUG 18	Simultaneous left and right truncation added to ANABSTR
NEWS	10	SEP 22	DIPPR file reloaded
NEWS	11	DEC 08	INPADOC: Legal Status data reloaded
NEWS	12	SEP 29	DISSABS now available on STN
NEWS	13	OCT 10	PCTFULL: Two new display fields added
NEWS	14	OCT 21	BIOSIS file reloaded and enhanced
NEWS	15	OCT 28	BIOSIS file segment of TOXCENTER reloaded and enhanced
NEWS	16	NOV 24	MSDS-CCOHS file reloaded
NEWS	17	DEC 08	CABA reloaded with left truncation
NEWS	18	DEC 08	IMS file names changed
NEWS	19	DEC 09	Experimental property data collected by CAS now available in REGISTRY
NEWS	20	DEC 09	STN Entry Date available for display in REGISTRY and CA/CAplus
NEWS	21	DEC 17	DGENE: Two new display fields added
NEWS	22	DEC 18	BIOTECHNO no longer updated
NEWS	23	DEC 19	CROPU no longer updated; subscriber discount no longer available

NEWS EXPRESS	NOVEMBER 14 CURRENT WINDOWS VERSION IS V6.01c, CURRENT MACINTOSH VERSION IS V6.0b(ENG) AND V6.0Jb(JP), AND CURRENT DISCOVER FILE IS DATED 23 SEPTEMBER 2003
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=> s zinc finger
L1       61216 ZINC FINGER

=> s l1 and binding
L2       32652 L1 AND BINDING

=> s nucleic acid binding protein
   6 FILES SEARCHED...
   7 FILES SEARCHED...
L3        2942 NUCLEIC ACID BINDING PROTEIN

=> ss cys2 (L) his2
L4         721 CYS2 (L) HIS2

=> s cys2 (L) his2
L5         721 CYS2 (L) HIS2

=> s l5 and l3
L6          38 L5 AND L3

=> d l6 ti abs ibib tot

L6   ANSWER 1 OF 38     MEDLINE on STN
TI   brlA requires both zinc fingers to induce development.
AB   Expression of the Aspergillus nidulans brlA gene induces a developmental
     pathway leading to the production of asexual spores.  We have introduced
     mutations into brlA that are expected to disrupt either or both
     Cys2-His2 Zn(II) coordination sites postulated to exist
```

in the BrlA polypeptide. The resultant brlA alleles fail to induce either the asexual reproductive pathway or the expression of development-specific genes. These data support the hypothesis that brlA encodes a **nucleic acid-binding protein** whose activity requires each of two zinc fingers.

ACCESSION NUMBER: 90205867 MEDLINE
DOCUMENT NUMBER: 90205867 PubMed ID: 2108321
TITLE: brlA requires both zinc fingers to induce development.
AUTHOR: Adams T H; Deising H; Timberlake W E
CORPORATE SOURCE: Department of Genetics, University of Georgia, Athens 30602.
CONTRACT NUMBER: GM37886 (NIGMS)
SOURCE: MOLECULAR AND CELLULAR BIOLOGY, (1990 Apr) 10 (4) 1815-7. Journal code: 8109087. ISSN: 0270-7306.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199005
ENTRY DATE: Entered STN: 19900601
Last Updated on STN: 20030130
Entered Medline: 19900502

L6 ANSWER 2 OF 38 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
TI BRLA REQUIRES BOTH ZINC FINGERS TO INDUCE DEVELOPMENT.
AB Expression of the Aspergillus nidulans brlA gene induces a developmental pathway leading to the production of asexual spores. We have introduced mutations into brlA that are expected to disrupt either or both **Cys2-His2** Zn(II) coordination sites postulated to exist in the brlA polypeptide. The resultant BrlA alleles fail to induce either the asexual reproductive pathway or the expression of development-specific genes. These data support the hypothesis that brlA encodes a **nucleic acid-binding protein** whose activity requires each of two zinc fingers.

ACCESSION NUMBER: 1990:218177 BIOSIS
DOCUMENT NUMBER: PREV199089115467; BA89:115467
TITLE: BRLA REQUIRES BOTH ZINC FINGERS TO INDUCE DEVELOPMENT.
AUTHOR(S): ADAMS T H [Reprint author]; DEISING H; TIMBERLAKE W E
CORPORATE SOURCE: DEP BIOL, TEXAS A AND M UNIV, COLL STN, TEXAS 77843, USA
SOURCE: Molecular and Cellular Biology, (1990) Vol. 10, No. 4, pp. 1815-1817.
CODEN: MCEBD4. ISSN: 0270-7306.
DOCUMENT TYPE: Article
FILE SEGMENT: BA
LANGUAGE: ENGLISH
ENTRY DATE: Entered STN: 10 May 1990
Last Updated on STN: 10 May 1990

L6 ANSWER 3 OF 38 WPIDS COPYRIGHT 2003 THOMSON DERWENT on STN
TI A polysome display-based technique for producing and selecting zinc finger nucleic acid binding proteins with desired binding characteristics.
AN 2000-376494 [32] WPIDS
AB WO 200027878 A UPAB: 20000725
NOVELTY - A method (I) for producing a zinc finger **nucleic acid binding protein**, comprising preparing a zinc finger according to design rules, varying the protein at one or more positions and selecting variants which bind to a target nucleic acid sequence by polysome display, is new.
USE - (I) is used to produce zinc finger binding proteins with desired binding characteristics.
ADVANTAGE - (I) comprises a polysome display technique which permits the isolation of binding polypeptides without resorting to phage display techniques.
Dwg.0/0

ACCESSION NUMBER: 2000-376494 [32] WPIDS
DOC. NO. CPI: C2000-113896
TITLE: A polysome display-based technique for producing and selecting zinc finger nucleic acid binding proteins with desired binding characteristics.
DERWENT CLASS: B04 C06 D16
INVENTOR(S): CHOO, Y; MOORE, M
PATENT ASSIGNEE(S): (GEND-N) GENDAQ LTD; (SANG-N) SANGAMO BIOSCIENCES INC; (CHOO-I) CHOO Y; (MOOR-I) MOORE M
COUNTRY COUNT: 88
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2000027878	A1	20000518	(200032)*	EN	46
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ TZ UG ZW					
W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZA ZW					
AU 2000010613	A	20000529	(200041)		
EP 1129109	A1	20010905	(200151)	EN	
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI					
US 2002064824	A1	20020530	(200240)		
JP 2002529067	W	20020910	(200274)		49
NZ 511564	A	20021025	(200274)		
AU 766572	B	20031016	(200380)		

APPLICATION DETAILS:

PATENT NO	KIND		APPLICATION	DATE
WO 2000027878	A1		WO 1999-GB3730	19991109
AU 2000010613	A		AU 2000-10613	19991109
EP 1129109	A1		EP 1999-954193	19991109
			WO 1999-GB3730	19991109
US 2002064824	A1	CIP of	WO 1999-GB3730	19991109
			US 2001-851271	20010508
JP 2002529067	W		WO 1999-GB3730	19991109
			JP 2000-581055	19991109
NZ 511564	A		NZ 1999-511564	19991109
			WO 1999-GB3730	19991109
AU 766572	B		AU 2000-10613	19991109

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 2000010613	A	Based on	WO 2000027878
EP 1129109	A1	Based on	WO 2000027878
JP 2002529067	W	Based on	WO 2000027878
NZ 511564	A	Based on	WO 2000027878
AU 766572	B	Previous Publ.	AU 2000010613
		Based on	WO 2000027878

PRIORITY APPLN. INFO: GB 1998-24544 19981109

L6 ANSWER 4 OF 38 WPIDS COPYRIGHT 2003 THOMSON DERWENT on STN
TI Rules for designing zinc finger nucleic acid binding proteins specific for any base quadruplet - relate bases in the quadruplet to specific amino acids in the alpha-helical binding motif, used to detect target nucleic acids, e.g. for identification of mutants and phosphorylation sites.

AN 1999-045309 [04] WPIDS
CR 1999-024577 [02]; 1999-024578 [02]
AB WO 9853060 A UPAB: 20020117
In a method for preparing a **nucleic acid binding protein** (I) of the **Cys2-His2** zinc finger (ZF) class, able to bind a nucleic acid quadruplet in a target sequence, binding to base 4 of the quadruplet by an alpha -helical ZF binding motif in (I) is determined as: (a) if base 4 is A, then position +6 in the helix is Gln and position ++2 is not Asp (++2 indicates a residue present in an adjacent, C-terminal ZF) and (b) if base 4 is C, then position +6 may be any residue provided ++2 is not Asp. Also new are (1) synthetic (I) designed according to these rules; (2) nucleic acid encoding such (I) and (3) host cells transformed with this nucleic acid.

(residues are numberred from the the first residue in the alpha helix e.g. +6 is the sixth residue, ++2 refers to residues present in an adjacent (C-terminal) zinc finger)

USE - (I) are used to detect target nucleic acids in a binding assay, e.g. for identification of mutants (they can differentiate between single bp changes in the target) or potential phosphorylation sites, and to characterise functional domains of a protein. (I) may also be used to create chimaeric restriction enzymes, by attaching (I) to a cleavage domain.

ADVANTAGE - The new method provides a more complete recognition code for interactions between ZF and nucleic acid, allowing design of (I) with absolute specificity for any selected target quadruplet. Particularly it allows design of (I) that bind to quadruplets that have a base other than G as the 5'-residue. The (I)-target interaction can be detected by ELISA (enzyme-linked immunosorbent assay), eliminating the need for gel electrophoresis and allowing automation of the assay.

Dwg.1B/7

ACCESSION NUMBER: 1999-045309 [04] WPIDS
CROSS REFERENCE: 1999-024577 [02]; 1999-024578 [02]
DOC. NO. CPI: C1999-014218
TITLE: Rules for designing zinc finger nucleic acid binding proteins specific for any base quadruplet - relate bases in the quadruplet to specific amino acids in the alpha-helical binding motif, used to detect target nucleic acids, e.g. for identification of mutants and phosphorylation sites.
DERWENT CLASS: B04 D16
INVENTOR(S): CHOO, Y; ISALAN, M; KLUG, A
PATENT ASSIGNEE(S): (MEDI-N) MEDICAL RES COUNCIL; (GEND-N) GENDAQ LTD
COUNTRY COUNT: 83
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 9853060	A1	19981126	(199904)*	EN	56
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SZ UG ZW					
W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH GM GW HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZW					
AU 9875426	A	19981211	(199917)		
EP 983351	A1	20000308	(200017)	EN	
R: AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE					
AU 732017	B	20010412	(200128)		
JP 2001527417 W		20011225	(200204)		55

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE

WO 9853060	A1	WO 1998-GB1516	19980526
AU 9875426	A	AU 1998-75426	19980526
EP 983351	A1	EP 1998-922967	19980526
		WO 1998-GB1516	19980526
AU 732017	B	AU 1998-75426	19980526
JP 2001527417	W	JP 1998-550158	19980526
		WO 1998-GB1516	19980526

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 9875426	A	Based on	WO 9853060
EP 983351	A1	Based on	WO 9853060
AU 732017	B	Previous Publ.	AU 9875426
		Based on	WO 9853060
JP 2001527417	W	Based on	WO 9853060

PRIORITY APPLN. INFO: GB 1997-10809 19970523

L6 ANSWER 5 OF 38 WPIDS COPYRIGHT 2003 THOMSON DERWENT on STN

TI Preparation of nucleic acid binding proteins - by designing protein sequences of a **Cys2-His2** zinc finger class based on a nucleic acid base triplet in a target nucleic acid sequence.

AN 1999-045308 [04] WPIDS

AB WO 9853059 A UPAB: 19990127

(A) A method is claimed for preparing a **nucleic acid binding protein** (NABP) of the **Cys2-His2** zinc finger class capable of binding to a nucleic acid base triplet in a target nucleic acid sequence, where binding to the 5' base of the triplet by an alpha -helical zinc finger nucleic acid binding motif in the protein is determined as follows: (a) if the 5' base in the triplet is A, then position +6 in the alpha -helix is Glu, Asn or Val; (b) if the 5' base in the triplet is C, then position +6 in the alpha -helix is Ser, Thr, Val, Ala, Glu or Asn.

Also claimed are:

(1) a method for preparing a NABP of the **Cys2-His2** zinc finger class capable of binding to a nucleic acid triplet in a target nucleic acid sequence, where binding to each base of the triplet by an alpha -helical zinc finger nucleic acid binding motif in the protein is determined as follows: (a) if the 5' base in the triplet is G, then position +6 in the alpha -helix is Arg or Lys; (b) if the 5' base in the triplet is A, then position +6 in the alpha -helix is Glu, Asn or Val; (c) if the 5' base in the triplet is T, then position +6 in the alpha -helix is Ser, Thr, Val or Lys; (d) if the 5' base in the triplet is C, then position +6 in the alpha -helix is Ser, Thr, Val Ala, Glu or Asn; (e) if the central base in the triplet is G, then position +3 in the alpha -helix is His; (f) if the central base in the triplet is A, then position +3 in the alpha -helix is Asn; (g) if the central base in the triplet is T, then position +3 in the alpha -helix is Ala, Ser or Val; provided that if it is Ala, then one of the residues at -1 or +6 is a small residue; (h) if the central base in the triplet is C, then position +3 in the alpha -helix is Ser, Asp, Glu, Leu, Thr or Val; (i) if the 3' base in the triplet is G, then position -1 in the alpha -helix is Arg; (j) if the 3' base in the triplet is A, then position -1 in the alpha -helix is Gln; (k) if the 3' base in the triplet is T, then position -1 in the alpha -helix is His or Thr; (l) if the 3' base in the triplet is C, then position -1 in the alpha -helix is Asp or His;

(2) a method for determining the presence of a target nucleic acid molecule (NAM), comprising: (a) preparing a NABP by a method as above which is specific for the target NAM; (b) exposing a test system comprising the target NAM to the NABP under conditions which promote binding, and removing any NABP which remains unbound; (c) detecting the presence of the NABP in the test system;

(3) a synthetic NABP whose design incorporates a method as in (A) or (1); (4) a nucleic acid encoding a NABP as in (3); (5) a host cell transformed with a nucleic acid as in (4);

USE - The methods can be used for designing a protein which is capable of binding to any predefined nucleic acid sequence. The NABPs can be used for the detection of target NAMs. They can also be used in gene therapy, e.g. for the delivery of functional genes into defective genes, or the delivery of nonsense nucleic acid to disrupt undesired nucleic acid.

Dwg.0/7

ACCESSION NUMBER: 1999-045308 [04] WPIDS
DOC. NO. CPI: C1999-014217
TITLE: Preparation of nucleic acid binding proteins - by designing protein sequences of a **Cys2-His2** zinc finger class based on a nucleic acid base triplet in a target nucleic acid sequence.
DERWENT CLASS: B04 D16
INVENTOR(S): CHOO, Y; ISALAN, M; KLUG, A
PATENT ASSIGNEE(S): (MEDI-N) MEDICAL RES COUNCIL
COUNTRY COUNT: 82
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 9853059	A1	19981126	(199904)*	EN	62

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SZ UG ZW
W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH GM GW HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZW

AU 9875424	A	19981211	(199917)

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 9853059	A1	WO 1998-GB1514	19980526
AU 9875424	A	AU 1998-75424	19980526

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 9875424	A	Based on	WO 9853059

PRIORITY APPLN. INFO: GB 1997-10807 19970523

L6 ANSWER 6 OF 38 WPIDS COPYRIGHT 2003 THOMSON DERWENT on STN
TI Preparing **Cys2-His2** zinc finger class nucleic acid binding proteins - capable of binding to a nucleic acid quadruplet, by mutating sites in model zinc finger domains according to a defined set of substitutions.
AN 1999-024578 [02] WPIDS
CR 1999-024577 [02]; 1999-045309 [04]
AB WO 9853058 A UPAB: 20000405
Preparing a **nucleic acid binding protein** (NBP) of the **Cys2-His2** zinc finger class capable of binding to a nucleic acid (NA) quadruplet in a target NA sequence is new. In the method, binding to base 4 of the quadruplet by an alpha -helical zinc finger NA binding motif is determined as follows: (a) if base 4 in the quadruplet is A, then position +6 in the alpha -helix is Glu, Asn or Val; and (b) if base 4 in the quadruplet is C, then position +6 in the alpha -helix is Ser, Thr, Val, Ala, Glu, or Asn. Preferably,

binding to base 4 is additionally determined as follows: (c) if base 4 of the quadruplet is G, then position +6 in the alpha -helix is Arg or Lys; and (d) if base 4 in the quadruplet is T, then position +6 in the alpha -helix is Ser, Thr, Val or Lys.

USE - The method is used for designing nucleic acid binding proteins which are useful in medicine (claimed). The proteins are specifically engineered to recognise particular nucleic acid sequences and as such are suitable for diagnosis of genetic disorders. The proteins can be used in the manufacture of chimeric restriction enzymes, in which a NA cleaving domain is fused to a NA binding domain comprising a zinc finger. Fusion protein comprising NBP and an integrase, e.g. viral integrase, can be used to target NA sequences in vivo. In gene therapy applications, the method may be targeted to the delivery of functional genes into defective genes, or the delivery of nonsense NA in order to disrupt undesired NA. Genes may also be delivered to known, repetitive stretches of nucleic acid, e.g. centromeres, together with an activating sequence such as an LCR. NBP can be specifically used to knockout cells having mutant proteins, e.g. mutant ras. They can also be used to modulate the action of transcription factors, e.g. the activity of HIV tat may be reduced by NBP specific for HIV TAR. NBP may also be coupled to toxic molecules, e.g. nucleases, which are capable of selectively destroying cells which comprise a mutation in their endogenous nucleic acid.

ADVANTAGE - The method provides a code of amino acid position bias which permits the selection of binding proteins against any nucleic acid target sequence. Every residue in a zinc finger nucleic acid binding motif which will bind specifically to a given nucleic acid quadruplet can be defined. When a marker protein is coexpressed with the binding protein, the requirement for gel electrophoresis is obviated, and so opens the way for automated nucleic acid diagnosis.

Dwg.0/8

ACCESSION NUMBER: 1999-024578 [02] WPIDS
CROSS REFERENCE: 1999-024577 [02]; 1999-045309 [04]
DOC. NO. CPI: C1999-007689
TITLE: Preparing **Cys2-His2** zinc finger class nucleic acid binding proteins - capable of binding to a nucleic acid quadruplet, by mutating sites in model zinc finger domains according to a defined set of substitutions.
DERWENT CLASS: B04 D16
INVENTOR(S): CHOO, Y; ISALAN, M; KLUG, A
PATENT ASSIGNEE(S): (MEDI-N) MEDICAL RES COUNCIL; (GEND-N) GENDAQ LTD
COUNTRY COUNT: 83
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 9853058	A1	19981126	(199902)*	EN	63
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SZ UG ZW					
W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH GM GW HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZW					
AU 9875423	A	19981211	(199917)		
EP 983350	A1	20000308	(200017)	EN	
R: AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE					

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 9853058	A1	WO 1998-GB1512	19980526
AU 9875423	A	AU 1998-75423	19980526
EP 983350	A1	EP 1998-922964	19980526

WO 1998-GB1512 19980526

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 9875423	A	Based on	WO 9853058
EP 983350	A1	Based on	WO 9853058

PRIORITY APPLN. INFO: GB 1997-10809 19970523

L6 ANSWER 7 OF 38 WPIDS COPYRIGHT 2003 THOMSON DERWENT on STN
TI New library of nucleic acid binding zinc finger polypeptide(s) - each polypeptide comprising more than one zinc finger which is partially randomised, useful for detecting a target nucleic acid sequence.
AN 1999-024577 [02] WPIDS
CR 1999-024578 [02]; 1999-045309 [04]
AB WO 9853057 A UPAB: 20020215
A zinc finger polypeptide library (I) in which each polypeptide comprises more than one zinc finger which has been at least partially randomised is new.

Also claimed are: (1) a set (II) of zinc finger polypeptide libraries which encode overlapping zinc finger polypeptides which may be assembled after selection to form a multifinger zinc finger polypeptide; and (2) a method of preparing a library of nucleic acid (NA) binding proteins of the **Cys2-His2** zinc finger class capable of binding to a target NA sequence.

USE - The method of (2) is useful for specifically engineering zinc finger proteins which can bind to particular nucleic acid targets. The resulting proteins can be used for determining the presence of a target nucleic acid (claimed). The proteins of the invention can be used in the manufacture of chimeric restriction enzymes, in which a NA cleaving domain is fused to a NA binding domain comprising a zinc finger. Fusion proteins comprising a binding protein and an integrase, e.g. viral integrase, can be used to target NA sequences in vivo. In gene therapy applications, the method may be targeted to the delivery of functional genes into defective genes, or the delivery of nonsense NA in order to disrupt undesired NA. Genes may also be delivered to known, repetitive stretches of nucleic acid, e.g. centromeres, together with an activating sequence such as an LCR. NA binding proteins can be specifically used to knock-out cells having mutant proteins, e.g. mutant ras. They can also be used to modulate the action of transcription factors, e.g. the activity of HIV tat may be reduced by binding proteins specific for HIV TAR. The new binding proteins may also be coupled to toxic molecules, e.g. nucleases, which are capable of selectively destroying cells which comprise a mutation in their endogenous nucleic acid. The products can be used in the treatment of infections.

ADVANTAGE - The invention provides a code of amino acid position bias which permits the selection of the library against any target nucleic acid sequence, and the production of a specific **nucleic acid binding protein**. Synergistic interactions between adjacent zinc fingers are taken into account, allowing the selection of any desired binding site. The invention allows the definition of every residue in a zinc finger nucleic acid binding motif which will bind specifically to a given nucleic acid quadruplet. When a marker protein is co-expressed with the binding protein, the requirement for gel electrophoresis is obviated, and so opens the way for automated nucleic acid diagnosis.

Dwg.0/6

ACCESSION NUMBER: 1999-024577 [02] WPIDS
CROSS REFERENCE: 1999-024578 [02]; 1999-045309 [04]
DOC. NO. CPI: C1999-007688
TITLE: New library of nucleic acid binding zinc finger polypeptide(s) - each polypeptide comprising more than

one zinc finger which is partially randomised, useful for detecting a target nucleic acid sequence.
DERWENT CLASS: B04 D16
INVENTOR(S): CHOO, Y; ISALAN, M; KLUG, A
PATENT ASSIGNEE(S): (MEDI-N) MEDICAL RES COUNCIL; (GEND-N) GENDAQ LTD
COUNTRY COUNT: 83
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 9853057	A1	19981126	(199902)*	EN	56
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SZ UG ZW					
W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH GM GW HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZW					
AU 9875422	A	19981211	(199917)		
EP 983349	A1	20000308	(200017)	EN	
R: AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE					
AU 737756	B	20010830	(200155)		
JP 2002502238	W	20020122	(200211)		56

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 9853057	A1	WO 1998-GB1510	19980526
AU 9875422	A	AU 1998-75422	19980526
EP 983349	A1	EP 1998-922963	19980526
		WO 1998-GB1510	19980526
AU 737756	B	AU 1998-75422	19980526
JP 2002502238	W	JP 1998-550153	19980526
		WO 1998-GB1510	19980526

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 9875422	A	Based on	WO 9853057
EP 983349	A1	Based on	WO 9853057
AU 737756	B	Previous Publ.	AU 9875422
		Based on	WO 9853057
JP 2002502238	W	Based on	WO 9853057

PRIORITY APPLN. INFO: GB 1997-10809 19970523

L6 ANSWER 8 OF 38 HCAPLUS COPYRIGHT 2003 ACS on STN
TI Recognition code for the design of synthetic nucleic acid-binding proteins
AB The invention provides a method for prepg. a **nucleic acid binding protein** of the **Cys2-His2** zinc finger class capable of binding to a target quadruplet nucleic acid sequence. A more complete code is provided which permits the selection of any nucleic acid sequence as the target sequence, and the design of a specific **nucleic acid-binding protein** which will bind thereto. Moreover, the invention provides a method by which a zinc finger protein specific for any given nucleic acid sequence may be designed and optimized. If bas 4 in the quadruplet is A, then position +6 in the .alpha.-helix is Gln and position ++2 is not Asp; and if base 4 in the quadruplet is C, then position +6 in the .alpha.-helix may be any residue, as long as position ++2 in the .alpha.-helix is not Asp. The recognition code is used to design (1) a protein whereby the target is the activating point mutation in codon 12 of the human EJ bladder carcinoma Ha-ras oncogene (GGC.fwdarw.GTC), and (2)

an anti-HIV zinc finger binding to the tat-specific sequence 5'-agagagctc-3'.

ACCESSION NUMBER: 1998:790665 HCAPLUS
DOCUMENT NUMBER: 130:35366
TITLE: Recognition code for the design of synthetic nucleic acid-binding proteins
INVENTOR(S): Choo, Yen; Klug, Aaron; Isalan, Mark
PATENT ASSIGNEE(S): Medical Research Council, UK
SOURCE: PCT Int. Appl., 57 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 3
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9853060	A1	19981126	WO 1998-GB1516	19980526
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
AU 9875426	A1	19981211	AU 1998-75426	19980526
AU 732017	B2	20010412		
EP 983351	A1	20000308	EP 1998-922967	19980526
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
JP 2001527417	T2	20011225	JP 1998-550158	19980526

PRIORITY APPLN. INFO.: GB 1997-10809 A 19970523
WO 1998-GB1516 W 19980526
OTHER SOURCE(S): MARPAT 130:35366
REFERENCE COUNT: 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 9 OF 38 HCAPLUS COPYRIGHT 2003 ACS on STN
TI Recognition code for the design of synthetic nucleic acid-binding proteins
AB The invention provides a method for prepg. a **nucleic acid binding protein** of the **Cys2-His2** zinc finger class capable of binding to a target triplet nucleic acid sequence. A more complete code is provided which permits the selection of any nucleic acid sequence as the target sequence, and the design of a specific **nucleic acid-binding protein** which will bind thereto. Moreover, the invention provides a method by which a zinc finger protein specific for any given nucleic acid sequence may be designed and optimized. Binding to the 5' base of the triplet by an .alpha.-helical zinc finger nucleic acid binding motif in the protein is detd. as follows: if the 5' base in the triplet is A, then position +6 in the .alpha.-helix is Glu, Asn or Val; if the 5' base in the triplet is C, then position +6 in the .alpha.-helix is Ser, Thr, Val, Ala, Glu or Asn. The recognition code is used to design (1) a protein whereby the target is the activating point mutation in codon 12 of the human EJ bladder carcinoma Ha-ras oncogene (GGC.fwdarw.GTC), (2) an anti-HIV zinc finger binding to the tat-specific sequence 5'-agagagctc-3', and (3) design of a zinc finger specific for an 8-bp palindrome (gcggccgc).

ACCESSION NUMBER: 1998:790664 HCAPLUS
DOCUMENT NUMBER: 130:35365
TITLE: Recognition code for the design of synthetic nucleic acid-binding proteins
INVENTOR(S): Choo, Yen; Klug, Aaron; Isalan, Mark

PATENT ASSIGNEE(S): Medical Research Council, UK
SOURCE: PCT Int. Appl., 63 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9853059	A1	19981126	WO 1998-GB1514	19980526
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
AU 9875424	A1	19981211	AU 1998-75424	19980526
PRIORITY APPLN. INFO.:			GB 1997-10807	19970523
			WO 1998-GB1514	19980526

OTHER SOURCE(S): MARPAT 130:35365
REFERENCE COUNT: 13 THERE ARE 13 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 10 OF 38 HCAPLUS COPYRIGHT 2003 ACS on STN
TI Recognition code for the design of synthetic nucleic acid-binding proteins
AB The invention provides a method for prepg. a **nucleic acid binding protein** of the **Cys2-His2** zinc finger class capable of binding to a target quadruplet nucleic acid sequence. Zinc finger binding sites are detd. by overlapping 4-bp subsites, and sequence specificity at the boundary between subsites arises from synergy between adjacent finger.s. A more complete code is provided which permits the selection of any nucleic acid sequence as the target sequence, and the design of a specific **nucleic acid-binding protein** which will bind thereto. Moreover, the invention provides a method by which a zinc finger protein specific for any given nucleic acid sequence may be designed and optimized. Binding to base 4 of the quadruplet by an .alpha.-helical zinc finger nucleic acid-binding motif in the protein is detd. as follows: if base 4 in the quadruplet is A, then position +6 in the .alpha.-helix is Glu, Asn, or Val; if base 4 in the quadruplet is C, then position +6 in the .alpha.-helix is Ser, Thr, Val, Ala, Glu, or Asn. The recognition code is used to design (1) a protein whereby the target is the activating point mutation in codon 12 of the human EJ bladder carcinoma Ha-ras oncogene (GGC.fwdarw.GTC), (2) an anti-HIV zinc finger binding to the tat-specific sequence 5'-agagagctc-3', and (3) design of a zinc finger specific for an 8-bp palindrome (gcggccgc).
ACCESSION NUMBER: 1998:790663 HCAPLUS
DOCUMENT NUMBER: 130:35364
TITLE: Recognition code for the design of synthetic nucleic acid-binding proteins
INVENTOR(S): Choo, Yen; Klug, Aaron; Isalan, Mark
PATENT ASSIGNEE(S): Medical Research Council, UK
SOURCE: PCT Int. Appl., 63 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 3
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE

WO 9853058 A1 19981126 WO 1998-GB1512 19980526
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG
AU 9875423 A1 19981211 AU 1998-75423 19980526
EP 983350 A1 20000308 EP 1998-922964 19980526
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI
PRIORITY APPLN. INFO.: GB 1997-10809 A 19970523
WO 1998-GB1512 W 19980526
OTHER SOURCE(S): MARPAT 130:35364
REFERENCE COUNT: 2 THERE ARE 2 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 11 OF 38 HCAPLUS COPYRIGHT 2003 ACS on STN
TI brlA requires both zinc fingers to induce development
AB Expression of the Aspergillus nidulans brlA gene induces a developmental pathway leading to the prodn. of asexual spores. Mutations were introduced into brlA that were expected to disrupt either or both **Cys2-His2** Zn(II) coordination sites postulated to exist in the BrlA polypeptide. The resultant brlA alleles fail to induce either the asexual reproductive pathway or the expression of development-specific genes. These data support the hypothesis that brlA encodes a **nucleic acid-binding protein** whose activity requires each of 2 zinc fingers.
ACCESSION NUMBER: 1990:175439 HCAPLUS
DOCUMENT NUMBER: 112:175439
TITLE: brlA requires both zinc fingers to induce development
AUTHOR(S): Adams, Thomas H.; Deising, Holger; Timberlake, William E.
CORPORATE SOURCE: Dep. Genet., Univ. Georgia, Athens, GA, 30602, USA
SOURCE: Molecular and Cellular Biology (1990), 10(4), 1815-17
CODEN: MCEBD4; ISSN: 0270-7306
DOCUMENT TYPE: Journal
LANGUAGE: English

L6 ANSWER 12 OF 38 USPATFULL on STN
TI Methods for modulating telomerase activity
AB The present invention relates to isolated or purified molecule(s) capable of binding to one or more of telomeric, G-quadruplex, or G-quartet nucleic acid(s).

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2003:232000 USPATFULL
TITLE: Methods for modulating telomerase activity
INVENTOR(S): Choo, Yen, London, UNITED KINGDOM
Isalan, Mark, London, UNITED KINGDOM
Patel, Sachin D., Mumbai, INDIA
Balasubramanian, Shankar, Cambridge, UNITED KINGDOM
Liu, Xiaohai, Cambridge, UNITED KINGDOM
PATENT ASSIGNEE(S): Gendaq Limited and Cambridge University Technical Services, Ltd. (non-U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2003162200	A1	20030828
APPLICATION INFO.:	US 2002-271708	A1	20021015 (10)

RELATED APPLN. INFO.: Continuation of Ser. No. US 2000-614679, filed on 12 Jul 2000, GRANTED, Pat. No. US 6492117

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: COOLEY GODWARD, LLP, 3000 EL CAMINO REAL, 5 PALO ALTO SQUARE, PALO ALTO, CA, 94306
NUMBER OF CLAIMS: 23
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 6 Drawing Page(s)
LINE COUNT: 1591
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 13 OF 38 USPATFULL on STN
TI Libraries of expressible gene sequences
AB The invention described herein comprises libraries of expressible gene sequences. Such gene sequences are contained on plasmid vectors designed to endow the expressed proteins with a number of useful features such as affinity purification tags, epitope tags, and the like. The expression vectors containing such gene sequences can be used to transfect cells for the production of recombinant proteins. A further aspect of the invention comprises methods of identifying binding partners for the products of such expressible gene sequences.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2003:194491 USPATFULL
TITLE: Libraries of expressible gene sequences
INVENTOR(S): Fernandez, Joseph Manuel, Carlsbad, CA, UNITED STATES
Heyman, John Alastair, Cardiff-by-the-Sea, CA, UNITED STATES
Hoeffler, James Paul, Carlsbad, CA, UNITED STATES
PATENT ASSIGNEE(S): INVITROGEN CORPORATION (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2003134302	A1	20030717
APPLICATION INFO.:	US 2002-210985	A1	20020801 (10)

RELATED APPLN. INFO.: Continuation of Ser. No. US 2001-3021, filed on 14 Nov 2001, PENDING Continuation of Ser. No. US 1999-285386, filed on 2 Apr 1999, ABANDONED

	NUMBER	DATE
PRIORITY INFORMATION:	US 1998-96981P	19980818 (60)
	US 1998-80626P	19980403 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: Lisa A. Haile, J.D., Ph.D., GRAY CARY WARE & FREIDENRICH LLP, Suite 1100, 4365 Executive Drive, San Diego, CA, 92121-2133
NUMBER OF CLAIMS: 40
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 1 Drawing Page(s)
LINE COUNT: 9810
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 14 OF 38 USPATFULL on STN
TI Nucleic acid binding polypeptides characterized by flexible linkers connected nucleic acid binding modules
AB We describe a method of producing a modified nucleic acid binding polypeptide, the method comprising the steps of: (a) providing a nucleic acid binding polypeptide comprising a plurality of nucleic acid binding modules; (b) selecting a first binding domain consisting of one or two contiguous nucleic acid binding modules; (c) selecting a second binding domain consisting of one or two contiguous nucleic acid binding modules; and (d) introducing a flexible linker sequence to link the first and second binding domains, the flexible linker sequence comprising five or

more amino acid residues. Use of structured linkers, alone or in combination with flexible linkers, is also disclosed.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	2003:173179 USPATFULL
TITLE:	Nucleic acid binding polypeptides characterized by flexible linkers connected nucleic acid binding modules
INVENTOR(S):	Choo, Yen, Cambridge, UNITED KINGDOM Klug, Aaron, Cambridge, UNITED KINGDOM Moore, Michael, Bucks, UNITED KINGDOM

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2003119023	A1	20030626	
APPLICATION INFO.:	US 2002-198677	A1	20020717	(10)
	WO 2001-GB202		20010119	

DOCUMENT TYPE:	Utility
FILE SEGMENT:	APPLICATION
LEGAL REPRESENTATIVE:	ROBINS & PASTERNAK LLP, Suite 180, 545 Middlefield Road, Menlo Park, CA, 94025
NUMBER OF CLAIMS:	51
EXEMPLARY CLAIM:	1
NUMBER OF DRAWINGS:	27 Drawing Page(s)
LINE COUNT:	3764

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 15 OF 38 USPATFULL on STN

TI Molecular switches

AB Provided herein are compositions comprising molecular switches and methods for identifying, selecting and using such molecular switches. Also provided are methods for the identification and use of ligand-dependent binding molecules and ligands.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	2003:133925 USPATFULL
TITLE:	Molecular switches
INVENTOR(S):	Choo, Yen, Cambridge, UNITED KINGDOM Ullman, Christopher Graeme, London, UNITED KINGDOM
PATENT ASSIGNEE(S):	Sangamo BioSciences, Inc., Richmond, CA (non-U.S. corporation)

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2003092010	A1	20030515	
APPLICATION INFO.:	US 2001-996484	A1	20011128	(9)

	NUMBER	DATE
PRIORITY INFORMATION:	WO 2000-GB2080	20000530
	GB 1999-12635	19990528
	GB 2000-1582	20000124

DOCUMENT TYPE:	Utility
FILE SEGMENT:	APPLICATION
LEGAL REPRESENTATIVE:	ROBINS & PASTERNAK LLP, 545 MIDDLEFIELD ROAD, SUITE 180, MENLO PARK, CA, 94025
NUMBER OF CLAIMS:	47
EXEMPLARY CLAIM:	1
NUMBER OF DRAWINGS:	3 Drawing Page(s)
LINE COUNT:	4056

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 16 OF 38 USPATFULL on STN

TI Libraries of expressible gene sequences

AB The invention described herein comprises libraries of expressible gene

sequences. Such gene sequences are contained on plasmid vectors designed to endow the expressed proteins with a number of useful features such as affinity purification tags, epitope tags, and the like. The expression vectors containing such gene sequences can be used to transfect cells for the production of recombinant proteins. A further aspect of the invention comprises methods of identifying binding partners for the products of such expressible gene sequences.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	2003:106252 USPATFULL
TITLE:	Libraries of expressible gene sequences
INVENTOR(S):	Fernandez, Joseph Manuel, Carlsbad, CA, UNITED STATES Heyman, John Alastair, Cardiff-by-the-Sea, CA, UNITED STATES Hoeffler, James Paul, Carlsbad, CA, UNITED STATES
PATENT ASSIGNEE(S):	INVITROGEN CORPORATION (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2003073163	A1	20030417
APPLICATION INFO.:	US 2001-3021	A1	20011114 (10)
RELATED APPLN. INFO.:	Continuation of Ser. No. US 1999-285386, filed on 2 Apr 1999, PENDING		

	NUMBER	DATE
PRIORITY INFORMATION:	US 1998-96981P	19980818 (60)
	US 1998-80626P	19980403 (60)

DOCUMENT TYPE:	Utility
FILE SEGMENT:	APPLICATION
LEGAL REPRESENTATIVE:	Lisa A. Haile, J.D., Ph.D., GRAY CARY WARE & FREIDENRICH LLP, Suite 1100, 4365 Executive Drive, San Diego, CA, 92121-2133
NUMBER OF CLAIMS:	40
EXEMPLARY CLAIM:	1
NUMBER OF DRAWINGS:	1 Drawing Page(s)
LINE COUNT:	9813

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 17 OF 38 USPATFULL on STN

TI Methods and compositions to modulate expression in plants

AB The invention relates to the field of plant and agricultural technology. More specifically, the invention relates to the use of zinc finger proteins and fusions of said proteins to regulate gene expression and metabolic pathways in plants.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	2003:52394 USPATFULL
TITLE:	Methods and compositions to modulate expression in plants
INVENTOR(S):	Barbas, Carlos F., III, Solana Beach, CA, UNITED STATES Stege, Justin T., San Diego, CA, UNITED STATES Guan, Xueni, San Diego, CA, UNITED STATES Dalmia, Bipin, San Diego, CA, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2003037355	A1	20030220
APPLICATION INFO.:	US 2001-765555	A1	20010119 (9)

	NUMBER	DATE
PRIORITY INFORMATION:	US 2000-177468P	20000121 (60)

DOCUMENT TYPE:	Utility

FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: MORRISON & FOERSTER LLP, 3811 VALLEY CENTRE DRIVE, SUITE 500, SAN DIEGO, CA, 92130-2332
NUMBER OF CLAIMS: 138
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 42 Drawing Page(s)
LINE COUNT: 4408
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 18 OF 38 USPATFULL on STN
TI Gene switches
AB Disclosed herein are methods and compositions relating to gene switches that use molecule capable of binding DNA sequences.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2003:32052 USPATFULL
TITLE: Gene switches
INVENTOR(S): Choo, Yen, Cambridge, UNITED KINGDOM
Ullman, Christopher Graeme, London, UNITED KINGDOM

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2003024006	A1	20030130	
APPLICATION INFO.:	US 2001-995973	A1	20011128	(9)

	NUMBER	DATE
PRIORITY INFORMATION:	WO 2000-GB2071	20000530
	GB 1999-12635	19990528
	GB 2000-1578	20000124

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: ROBINS & PASTERNAK LLP, 545 MIDDLEFIELD ROAD, SUITE 180, MENLO PARK, CA, 94025
NUMBER OF CLAIMS: 26
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 3 Drawing Page(s)
LINE COUNT: 3752
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 19 OF 38 USPATFULL on STN
TI Zinc finger polypeptides capable of binding DNA quadruplexes
AB The present invention relates to isolated or purified molecule(s) capable of binding to one or more of telomeric, G-quadruplex, or G-quartet nucleic acid(s).

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2002:325836 USPATFULL
TITLE: Zinc finger polypeptides capable of binding DNA quadruplexes
INVENTOR(S): Choo, Yen, London, UNITED KINGDOM
Isalan, Mark, London, UNITED KINGDOM
Patel, Sachin D., Mumbai, INDIA
Balasubramanian, Shhankar, Cambridge, UNITED KINGDOM
Liu, Xiaohai, Cambridge, UNITED KINGDOM
PATENT ASSIGNEE(S): Gendaq Limited, London, UNITED KINGDOM (non-U.S. corporation)

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 6492117	B1	20021210	
APPLICATION INFO.:	US 2000-614679		20000712	(9)

DOCUMENT TYPE: Utility
FILE SEGMENT: GRANTED

PRIMARY EXAMINER: Horlick, Kenneth R.
ASSISTANT EXAMINER: Strzelecka, Teresa
LEGAL REPRESENTATIVE: Robins & Pasternak LLP, Brennan, Sean M.
NUMBER OF CLAIMS: 22
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 10 Drawing Figure(s); 6 Drawing Page(s)
LINE COUNT: 1708
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 20 OF 38 USPATFULL on STN
TI Screening system for zinc finger polypeptides for a desired binding ability
AB This invention relates to a method for producing a zinc finger **nucleic acid binding protein** comprising preparing a zinc finger protein according design rules, varying the protein at one or more positions, and selecting variants which bind to a target nucleic acid sequence by polysome display.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2002:126312 USPATFULL
TITLE: Screening system for zinc finger polypeptides for a desired binding ability
INVENTOR(S): Choo, Yen, Cambridge, UNITED KINGDOM
Moore, Michael, Amersham Bucks, UNITED KINGDOM

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2002064824	A1	20020530
APPLICATION INFO.:	US 2001-851271	A1	20010508 (9)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. WO 1999-GB3730, filed on 9 Nov 1999, UNKNOWN

	NUMBER	DATE
PRIORITY INFORMATION:	GB 1998-24544	19981109

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: FROMMER LAWRENCE & HAUG LLP, 745 Fifth Avenue, New York, NY, 10151
NUMBER OF CLAIMS: 13
EXEMPLARY CLAIM: 1
LINE COUNT: 1356
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L6 ANSWER 21 OF 38 DGENE COPYRIGHT 2003 THOMSON DERWENT on STN
TI Preparing **Cys2-His2** zinc finger class nucleic acid binding proteins - capable of binding to a nucleic acid quadruplet, by mutating sites in model zinc finger domains according to a defined set of substitutions
AN AAW87558 Protein DGENE
AB The present sequence represents a consensus zinc finger used in the **nucleic acid binding protein** (NBP) of the **Cys2-His2** zinc finger class. The NBP is capable of binding to a nucleic acid quadruplet in a target sequence. The specification describers a method of preparing the NBP. The method is used for designing nucleic acid binding proteins which are useful in medicine. The proteins are specifically engineered to recognise particular nucleic acid sequences and as such are suitable for diagnosis of genetic disorders. The proteins can be used in the manufacture of chimeric restriction enzymes, in which a nucleic acid cleaving domain is fused to a nucleic acid binding domain comprising a zinc finger. Fusion protein comprising NBP and an integrase, e.g. viral integrase, can be used to target nucleic acid sequences in vivo. In gene therapy applications, the method may be targeted to the delivery of functional

genes into defective genes, or the delivery of nonsense nucleic acid in order to disrupt undesired nucleic acid. Genes may also be delivered to known, repetitive stretches of nucleic acid, e.g. centromeres, together with an activating sequence such as an LCR. NBP can be specifically used to knockout cells having mutant proteins e.g. mutant ras. They can also be used to modulate the action of transcription factors, e.g. the activity of HIV tat may be reduced by NBP specific for HIV TAR. NBP may also be coupled to toxic molecules, e.g. nucleases, which are capable of selectively destroying cells which comprise a mutation in their endogenous nucleic acid.

ACCESSION NUMBER: AAW87558 Protein DGENE
TITLE: Preparing **Cys2-His2** zinc finger class nucleic acid binding proteins - capable of binding to a nucleic acid quadruplet, by mutating sites in model zinc finger domains according to a defined set of substitutions
INVENTOR: Choo Y; Isalan M; Klug A
PATENT ASSIGNEE: (MEDI-N)MEDICAL RES COUNCIL.
PATENT INFO: WO 9853058 A1 19981126 64p
APPLICATION INFO: WO 1998-GB1512 19980526
PRIORITY INFO: GB 1997-10809 19970523
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: 1999-024578 [02]
DESCRIPTION: Consensus zinc finger used in the NBP of the invention.

L6 ANSWER 22 OF 38 DGENE COPYRIGHT 2003 THOMSON DERWENT on STN
TI Preparing **Cys2-His2** zinc finger class nucleic acid binding proteins - capable of binding to a nucleic acid quadruplet, by mutating sites in model zinc finger domains according to a defined set of substitutions
AN AAW87557 Protein DGENE
AB The present sequence represents a **nucleic acid binding protein** (NBP) of the **Cys2-His2** zinc finger class. The NBP is capable of binding to a nucleic acid quadruplet in a target sequence. The specification describers a method of preparing the NBP. The method is used for designing nucleic acid binding proteins which are useful in medicine. The proteins are specifically engineered to recognise particular nucleic acid sequences and as such are suitable for diagnosis of genetic disorders. The proteins can be used in the manufacture of chimeric restriction enzymes, in which a nucleic acid cleaving domain is fused to a nucleic acid binding domain comprising a zinc finger. Fusion protein comprising NBP and an integrase, e.g. viral integrase, can be used to target nucleic acid sequences in vivo. In gene therapy applications, the method may be targeted to the delivery of functional genes into defective genes, or the delivery of nonsense nucleic acid in order to disrupt undesired nucleic acid. Genes may also be delivered to known, repetitive stretches of nucleic acid, e.g. centromeres, together with an activating sequence such as an LCR. NBP can be specifically used to knockout cells having mutant proteins e.g. mutant ras. They can also be used to modulate the action of transcription factors, e.g. the activity of HIV tat may be reduced by NBP specific for HIV TAR. NBP may also be coupled to toxic molecules, e.g. nucleases, which are capable of selectively destroying cells which comprise a mutation in their endogenous nucleic acid.

ACCESSION NUMBER: AAW87557 Protein DGENE
TITLE: Preparing **Cys2-His2** zinc finger class nucleic acid binding proteins - capable of binding to a nucleic acid quadruplet, by mutating sites in model zinc finger domains according to a defined set of substitutions
INVENTOR: Choo Y; Isalan M; Klug A
PATENT ASSIGNEE: (MEDI-N)MEDICAL RES COUNCIL.
PATENT INFO: WO 9853058 A1 19981126 64p
APPLICATION INFO: WO 1998-GB1512 19980526
PRIORITY INFO: GB 1997-10809 19970523

DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: 1999-024578 [02]
CROSS REFERENCES: N-PSDB: AAV83634
DESCRIPTION: A **nucleic acid binding protein**.

L6 ANSWER 23 OF 38 DGENE COPYRIGHT 2003 THOMSON DERWENT on STN
TI Preparing **Cys2-His2** zinc finger class nucleic acid binding proteins - capable of binding to a nucleic acid quadruplet, by mutating sites in model zinc finger domains according to a defined set of substitutions
AN AAW87559 Protein DGENE
AB The present sequence represents a consensus zinc finger used in the **nucleic acid binding protein** (NBP) of the **Cys2-His2** zinc finger class. The NBP is capable of binding to a nucleic acid quadruplet in a target sequence. The specification describers a method of preparing the NBP. The method is used for designing nucleic acid binding proteins which are useful in medicine. The proteins are specifically engineered to recognise particular nucleic acid sequences and as such are suitable for diagnosis of genetic disorders. The proteins can be used in the manufacture of chimeric restriction enzymes, in which a nucleic acid cleaving domain is fused to a nucleic acid binding domain comprising a zinc finger. Fusion protein comprising NBP and an integrase, e.g. viral integrase, can be used to target nucleic acid sequences in vivo. In gene therapy applications, the method may be targeted to the delivery of functional genes into defective genes, or the delivery of nonsense nucleic acid in order to disrupt undesired nucleic acid. Genes may also be delivered to known, repetitive stretches of nucleic acid, e.g. centromeres, together with an activating sequence such as an LCR. NBP can be specifically used to knockout cells having mutant proteins e.g. mutant ras. They can also be used to modulate the action of transcription factors, e.g. the activity of HIV tat may be reduced by NBP specific for HIV TAR. NBP may also be coupled to toxic molecules, e.g. nucleases, which are capable of selectively destroying cells which comprise a mutation in their endogenous nucleic acid.
ACCESSION NUMBER: AAW87559 Protein DGENE
TITLE: Preparing **Cys2-His2** zinc finger class nucleic acid binding proteins - capable of binding to a nucleic acid quadruplet, by mutating sites in model zinc finger domains according to a defined set of substitutions
INVENTOR: Choo Y; Isalan M; Klug A
PATENT ASSIGNEE: (MEDI-N)MEDICAL RES COUNCIL.
PATENT INFO: WO 9853058 A1 19981126 64p
APPLICATION INFO: WO 1998-GB1512 19980526
PRIORITY INFO: GB 1997-10809 19970523
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: 1999-024578 [02]
DESCRIPTION: Consensus zinc finger used in the NBP of the invention.

L6 ANSWER 24 OF 38 DGENE COPYRIGHT 2003 THOMSON DERWENT on STN
TI New library of nucleic acid binding zinc finger polypeptide(s) - each polypeptide comprising more than one zinc finger which is partially randomised, useful for detecting a target nucleic acid sequence
AN AAW87789 peptide DGENE
AB The present sequence represents a spacer sequence used in the zinc finger polypeptide library of the invention. Each polypeptide of the library comprises more than one zinc finger which has been at least partially randomised. Zinc finger proteins bind to particular nucleic acid targets. The proteins can be used for determining the presence of a target nucleic acid. The proteins of the invention can be used in the manufacture of chimeric restriction enzymes, in which a nucleic acid cleaving domain is

fused to a nucleic acid binding domain comprising a zinc finger. Fusion proteins comprising a binding protein and an integrase, e.g. viral integrase, can be used to target nucleic acid sequences in vivo. In gene therapy applications, the method may be targeted to the delivery of functional genes into defective genes, or the delivery of nonsense nucleic acid. Genes may also be delivered to known repetitive stretches of nucleic acid e.g. centromeres, together with an activating sequence such as an LCR. Nucleic acid binding proteins can be specifically used to knock-out cells having mutant proteins, e.g. mutant ras. They can also be used to modulate the action of transcription factors, e.g. the activity of HIV tat may be reduced by binding proteins specific for HIV TAR. The new binding proteins may also be coupled to toxic molecules, e.g. nucleases, which are capable of selectively destroying cells which comprise a mutation in their endogenous nucleic acid. The products can be used in the treatment of infections.

ACCESSION NUMBER: AAW87789 peptide DGENE
TITLE: New library of nucleic acid binding zinc finger polypeptide(s) - each polypeptide comprising more than one zinc finger which is partially randomised, useful for detecting a target nucleic acid sequence
INVENTOR: Choo Y; Isalan M; Klug A
PATENT ASSIGNEE: (MEDI-N)MEDICAL RES COUNCIL.
PATENT INFO: WO 9853057 A1 19981126 56p
APPLICATION INFO: WO 1998-GB1510 19980526
PRIORITY INFO: GB 1997-10809 19970523
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: 1999-024577 [02]
DESCRIPTION: Spacer sequence of the zinc finger library.

L6 ANSWER 25 OF 38 DGENE COPYRIGHT 2003 THOMSON DERWENT on STN
TI New library of nucleic acid binding zinc finger polypeptide(s) - each polypeptide comprising more than one zinc finger which is partially randomised, useful for detecting a target nucleic acid sequence
AN AAW87700 peptide DGENE
AB The present sequence represents leader sequence attached to zinc fingers of the invention. The zinc fingers are used to create a zinc finger polypeptide library in which each polypeptide comprises more than one zinc finger which has been at least partially randomised. Zinc finger proteins bind to particular nucleic acid targets. The proteins can be used for determining the presence of a target nucleic acid. The proteins of the invention can be used in the manufacture of chimeric restriction enzymes, in which a nucleic acid cleaving domain is fused to a nucleic acid binding domain comprising a zinc finger. Fusion proteins comprising a binding protein and an integrase, e.g. viral integrase, can be used to target nucleic acid sequences in vivo. In gene therapy applications, the method may be targeted to the delivery of functional genes into defective genes, or the delivery of nonsense nucleic acid. Genes may also be delivered to known repetitive stretches of nucleic acid e.g. centromeres, together with an activating sequence such as an LCR. Nucleic acid binding proteins can be specifically used to knock-out cells having mutant proteins, e.g. mutant ras. They can also be used to modulate the action of transcription factors, e.g. the activity of HIV tat may be reduced by binding proteins specific for HIV TAR. The new binding proteins may also be coupled to toxic molecules, e.g. nucleases, which are capable of selectively destroying cells which comprise a mutation in their endogenous nucleic acid. The products can be used in the treatment of infections.

ACCESSION NUMBER: AAW87700 peptide DGENE
TITLE: New library of nucleic acid binding zinc finger polypeptide(s) - each polypeptide comprising more than one zinc finger which is partially randomised, useful for detecting a target nucleic acid sequence
INVENTOR: Choo Y; Isalan M; Klug A

PATENT ASSIGNEE: (MEDI-N)MEDICAL RES COUNCIL.
PATENT INFO: WO 9853057 A1 19981126 56p
APPLICATION INFO: WO 1998-GB1510 19980526
PRIORITY INFO: GB 1997-10809 19970523
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: 1999-024577 [02]
DESCRIPTION: Leader sequence attached to zinc fingers of the invention.

L6 ANSWER 26 OF 38 DGENE COPYRIGHT 2003 THOMSON DERWENT on STN
TI New library of nucleic acid binding zinc finger polypeptide(s) - each polypeptide comprising more than one zinc finger which is partially randomised, useful for detecting a target nucleic acid sequence
AN AAW87698 peptide DGENE
AB The present sequence represents a consensus zinc finger sequence of the **Cys2-His2** zinc finger class. It is used to create a zinc finger polypeptide library in which each polypeptide comprises more than one zinc finger which has been at least partially randomised. Zinc finger proteins bind to particular nucleic acid targets. The proteins can be used for determining the presence of a target nucleic acid. The proteins of the invention can be used in the manufacture of chimeric restriction enzymes, in which a nucleic acid cleaving domain is fused to a nucleic acid binding domain comprising a zinc finger. Fusion proteins comprising a binding protein and an integrase, e.g. viral integrase, can be used to target nucleic acid sequences in vivo. In gene therapy applications, the method may be targeted to the delivery of functional genes into defective genes, or the delivery of nonsense nucleic acid. Genes may also be delivered to known repetitive stretches of nucleic acid e.g. centromeres, together with an activating sequence such as an LCR. Nucleic acid binding proteins can be specifically used to knock-out cells having mutant proteins, e.g. mutant ras. They can also be used to modulate the action of transcription factors, e.g. the activity of HIV tat may be reduced by binding proteins specific for HIV TAR. The new binding proteins may also be coupled to toxic molecules, e.g. nucleases, which are capable of selectively destroying cells which comprise a mutation in their endogenous nucleic acid. The products can be used in the treatment of infections.
ACCESSION NUMBER: AAW87698 peptide DGENE
TITLE: New library of nucleic acid binding zinc finger polypeptide(s) - each polypeptide comprising more than one zinc finger which is partially randomised, useful for detecting a target nucleic acid sequence
INVENTOR: Choo Y; Isalan M; Klug A
PATENT ASSIGNEE: (MEDI-N)MEDICAL RES COUNCIL.
PATENT INFO: WO 9853057 A1 19981126 56p
APPLICATION INFO: WO 1998-GB1510 19980526
PRIORITY INFO: GB 1997-10809 19970523
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: 1999-024577 [02]
DESCRIPTION: Consensus zinc finger sequence of the **Cys2-His2** zinc finger class.

L6 ANSWER 27 OF 38 DGENE COPYRIGHT 2003 THOMSON DERWENT on STN
TI New library of nucleic acid binding zinc finger polypeptide(s) - each polypeptide comprising more than one zinc finger which is partially randomised, useful for detecting a target nucleic acid sequence
AN AAW87699 peptide DGENE
AB The present sequence represents a consensus zinc finger sequence of the **Cys2-His2** zinc finger class. It is used to create a zinc finger polypeptide library in which each polypeptide comprises more than one zinc finger which has been at least partially randomised. Zinc finger proteins bind to particular nucleic acid targets. The proteins can be used for determining the presence of a target nucleic acid. The

proteins of the invention can be used in the manufacture of chimeric restriction enzymes, in which a nucleic acid cleaving domain is fused to a nucleic acid binding domain comprising a zinc finger. Fusion proteins comprising a binding protein and an integrase, e.g. viral integrase, can be used to target nucleic acid sequences in vivo. In gene therapy applications, the method may be targeted to the delivery of functional genes into defective genes, or the delivery of nonsense nucleic acid. Genes may also be delivered to known repetitive stretches of nucleic acid e.g. centromeres, together with an activating sequence such as an LCR. Nucleic acid binding proteins can be specifically used to knock-out cells having mutant proteins, e.g. mutant ras. They can also be used to modulate the action of transcription factors, e.g. the activity of HIV tat may be reduced by binding proteins specific for HIV TAR. The new binding proteins may also be coupled to toxic molecules, e.g. nucleases, which are capable of selectively destroying cells which comprise a mutation in their endogenous nucleic acid. The products can be used in the treatment of infections.

ACCESSION NUMBER: AAW87699 peptide DGENE
TITLE: New library of nucleic acid binding zinc finger polypeptide(s) - each polypeptide comprising more than one zinc finger which is partially randomised, useful for detecting a target nucleic acid sequence
INVENTOR: Choo Y; Isalan M; Klug A
PATENT ASSIGNEE: (MEDI-N)MEDICAL RES COUNCIL.
PATENT INFO: WO 9853057 A1 19981126 56p
APPLICATION INFO: WO 1998-GB1510 19980526
PRIORITY INFO: GB 1997-10809 19970523
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: 1999-024577 [02]
DESCRIPTION: Consensus zinc finger sequence of the **Cys2-His2** zinc finger class.

L6 ANSWER 28 OF 38 DGENE COPYRIGHT 2003 THOMSON DERWENT on STN
TI Preparation of nucleic acid binding proteins - by designing protein sequences of a **Cys2-His2** zinc finger class based on a nucleic acid base triplet in a target nucleic acid sequence
AN AAW84303 Peptide DGENE
AB The present sequence represents a consensus zinc finger sequence of the invention. The specification describes a method for preparing a **nucleic acid binding protein** (NABP) of the **Cys2-His2** zinc finger class capable of binding to a nucleic acid base triplet in a target nucleic acid sequence. Binding to the 5' base of the triplet by an alpha-helical zinc finger nucleic acid binding motif in the protein is determined as follows: (a) if the 5' base in the triplet is A, then position +6 in the alpha -helix is Glu, Asn or Val; (b) if the 5' base in the triplet is C, then position +6 in the alpha-helix is Ser, Thr, Val, Ala, Glu or Asn. The methods can be used for designing a protein which is capable of binding to any predefined nucleic acid sequence. The NABPs can be used for the detection of target nucleic acid molecules. They can also be used in gene therapy, e.g. for the delivery of functional genes into defective genes, or the delivery of nonsense nucleic acid to disrupt undesired nucleic acid.

ACCESSION NUMBER: AAW84303 Peptide DGENE
TITLE: Preparation of nucleic acid binding proteins - by designing protein sequences of a **Cys2-His2** zinc finger class based on a nucleic acid base triplet in a target nucleic acid sequence
INVENTOR: Choo Y; Isalan M; Klug A
PATENT ASSIGNEE: (MEDI-N)MEDICAL RES COUNCIL.
PATENT INFO: WO 9853059 A1 19981126 62p
APPLICATION INFO: WO 1998-GB1514 19980526
PRIORITY INFO: GB 1997-10807 19970523
DOCUMENT TYPE: Patent

LANGUAGE: English
OTHER SOURCE: 1999-045308 [04]
DESCRIPTION: Consensus zinc finger sequence.

L6 ANSWER 29 OF 38 DGENE COPYRIGHT 2003 THOMSON DERWENT on STN
TI Preparation of nucleic acid binding proteins - by designing protein sequences of a **Cys2-His2** zinc finger class based on a nucleic acid base triplet in a target nucleic acid sequence
AN AAW84301 Peptide DGENE
AB The present sequence represents a linker used in the zinc fingers of the invention. The specification describes a method for preparing a **nucleic acid binding protein** (NABP) of the **Cys2-His2** zinc finger class capable of binding to a nucleic acid base triplet in a target nucleic acid sequence. Binding to the 5' base of the triplet by an alpha-helical zinc finger nucleic acid binding motif in the protein is determined as follows: (a) if the 5' base in the triplet is A, then position +6 in the alpha -helix is Glu, Asn or Val; (b) if the 5' base in the triplet is C, then position +6 in the alpha-helix is Ser, Thr, Val, Ala, Glu or Asn. The methods can be used for designing a protein which is capable of binding to any predefined nucleic acid sequence. The NABPs can be used for the detection of target nucleic acid molecules. They can also be used in gene therapy, e.g. for the delivery of functional genes into defective genes, or the delivery of nonsense nucleic acid to disrupt undesired nucleic acid.
ACCESSION NUMBER: AAW84301 Peptide DGENE
TITLE: Preparation of nucleic acid binding proteins - by designing protein sequences of a **Cys2-His2** zinc finger class based on a nucleic acid base triplet in a target nucleic acid sequence
INVENTOR: Choo Y; Isalan M; Klug A
PATENT ASSIGNEE: (MEDI-N)MEDICAL RES COUNCIL.
PATENT INFO: WO 9853059 A1 19981126 62p
APPLICATION INFO: WO 1998-GB1514 19980526
PRIORITY INFO: GB 1997-10807 19970523
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: 1999-045308 [04]
DESCRIPTION: Linker used in the zinc fingers of the invention.

L6 ANSWER 30 OF 38 DGENE COPYRIGHT 2003 THOMSON DERWENT on STN
TI Preparation of nucleic acid binding proteins - by designing protein sequences of a **Cys2-His2** zinc finger class based on a nucleic acid base triplet in a target nucleic acid sequence
AN AAW84302 Peptide DGENE
AB The present sequence represents a consensus zinc finger sequence of the invention. The specification describes a method for preparing a **nucleic acid binding protein** (NABP) of the **Cys2-His2** zinc finger class capable of binding to a nucleic acid base triplet in a target nucleic acid sequence. Binding to the 5' base of the triplet by an alpha-helical zinc finger nucleic acid binding motif in the protein is determined as follows: (a) if the 5' base in the triplet is A, then position +6 in the alpha -helix is Glu, Asn or Val; (b) if the 5' base in the triplet is C, then position +6 in the alpha-helix is Ser, Thr, Val, Ala, Glu or Asn. The methods can be used for designing a protein which is capable of binding to any predefined nucleic acid sequence. The NABPs can be used for the detection of target nucleic acid molecules. They can also be used in gene therapy, e.g. for the delivery of functional genes into defective genes, or the delivery of nonsense nucleic acid to disrupt undesired nucleic acid.
ACCESSION NUMBER: AAW84302 Peptide DGENE
TITLE: Preparation of nucleic acid binding proteins - by designing protein sequences of a **Cys2-His2** zinc finger class based on a nucleic acid base triplet in a target nucleic acid sequence

INVENTOR: Choo Y; Isalan M; Klug A
PATENT ASSIGNEE: (MEDI-N)MEDICAL RES COUNCIL.
PATENT INFO: WO 9853059 A1 19981126 62p
APPLICATION INFO: WO 1998-GB1514 19980526
PRIORITY INFO: GB 1997-10807 19970523
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: 1999-045308 [04]
DESCRIPTION: Consensus zinc finger sequence.

L6 ANSWER 31 OF 38 DGENE COPYRIGHT 2003 THOMSON DERWENT on STN
TI Preparation of nucleic acid binding proteins - by designing protein sequences of a **Cys2-His2** zinc finger class based on a nucleic acid base triplet in a target nucleic acid sequence
AN AAW84300 Peptide DGENE
AB The present sequence represents a linker used in the zinc fingers of the invention. The specification describes a method for preparing a **nucleic acid binding protein** (NABP) of the **Cys2-His2** zinc finger class capable of binding to a nucleic acid base triplet in a target nucleic acid sequence. Binding to the 5' base of the triplet by an alpha-helical zinc finger nucleic acid binding motif in the protein is determined as follows: (a) if the 5' base in the triplet is A, then position +6 in the alpha -helix is Glu, Asn or Val; (b) if the 5' base in the triplet is C, then position +6 in the alpha-helix is Ser, Thr, Val, Ala, Glu or Asn. The methods can be used for designing a protein which is capable of binding to any predefined nucleic acid sequence. The NABPs can be used for the detection of target nucleic acid molecules. They can also be used in gene therapy, e.g. for the delivery of functional genes into defective genes, or the delivery of nonsense nucleic acid to disrupt undesired nucleic acid.
ACCESSION NUMBER: AAW84300 Peptide DGENE
TITLE: Preparation of nucleic acid binding proteins - by designing protein sequences of a **Cys2-His2** zinc finger class based on a nucleic acid base triplet in a target nucleic acid sequence
INVENTOR: Choo Y; Isalan M; Klug A
PATENT ASSIGNEE: (MEDI-N)MEDICAL RES COUNCIL.
PATENT INFO: WO 9853059 A1 19981126 62p
APPLICATION INFO: WO 1998-GB1514 19980526
PRIORITY INFO: GB 1997-10807 19970523
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: 1999-045308 [04]
DESCRIPTION: Linker used in the zinc fingers of the invention.

L6 ANSWER 32 OF 38 DGENE COPYRIGHT 2003 THOMSON DERWENT on STN
TI Preparation of nucleic acid binding proteins - by designing protein sequences of a **Cys2-His2** zinc finger class based on a nucleic acid base triplet in a target nucleic acid sequence
AN AAW84299 Protein DGENE
AB The present sequence encodes an anti-HIV zinc finger. The zinc finger was made to exemplify the invention. THe specification describes a method for preparing a **nucleic acid binding protein** (NABP) of the **Cys2-His2** zinc finger class capable of binding to a nucleic acid base triplet in a target nucleic acid sequence. Binding to the 5' base of the triplet by an alpha-helical zinc finger nucleic acid binding motif in the protein is determined as follows: (a) if the 5' base in the triplet is A, then position +6 in the alpha -helix is Glu, Asn or Val; (b) if the 5' base in the triplet is C, then position +6 in the alpha -helix is Ser, Thr, Val, Ala, Glu or Asn. The methods can be used for designing a protein which is capable of binding to any predefined nucleic acid sequence. The NABPs can be used for the detection of target nucleic acid molecules. They can also be used in gene therapy, e.g. for the delivery of functional genes into

defective genes, or the delivery of nonsense nucleic acid to disrupt undesired nucleic acid.

ACCESSION NUMBER: AAW84299 Protein DGENE
TITLE: Preparation of nucleic acid binding proteins - by designing protein sequences of a **Cys2-His2** zinc finger class based on a nucleic acid base triplet in a target nucleic acid sequence
INVENTOR: Choo Y; Isalan M; Klug A
PATENT ASSIGNEE: (MEDI-N)MEDICAL RES COUNCIL.
PATENT INFO: WO 9853059 A1 19981126 62p
APPLICATION INFO: WO 1998-GB1514 19980526
PRIORITY INFO: GB 1997-10807 19970523
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: 1999-045308 [04]
CROSS REFERENCES: N-PSDB: AAV99467
DESCRIPTION: An anti-HIV zinc finger.

L6 ANSWER 33 OF 38 DGENE COPYRIGHT 2003 THOMSON DERWENT on STN
TI Preparation of nucleic acid binding proteins - by designing protein sequences of a **Cys2-His2** zinc finger class based on a nucleic acid base triplet in a target nucleic acid sequence
AN AAW84306 Peptide DGENE
AB The present sequence represents finger 1 of a **nucleic acid binding protein** (NABP) specific for a G12V mutant ras oncogene. The specification describes a method for preparing a NABP of the **Cys2-His2** zinc finger class capable of binding to a nucleic acid base triplet in a target nucleic acid sequence. Binding to the 5' base of the triplet by an alpha-helical zinc finger nucleic acid binding motif in the protein is determined as follows: (a) if the 5' base in the triplet is A, then position +6 in the alpha -helix is Glu, Asn or Val; (b) if the 5' base in the triplet is C, then position +6 in the alpha-helix is Ser, Thr, Val, Ala, Glu or Asn. The methods can be used for designing a protein which is capable of binding to any predefined nucleic acid sequence. The NABPs can be used for the detection of target nucleic acid molecules. They can also be used in gene therapy, e.g. for the delivery of functional genes into defective genes, or the delivery of nonsense nucleic acid to disrupt undesired nucleic acid.

ACCESSION NUMBER: AAW84306 Peptide DGENE
TITLE: Preparation of nucleic acid binding proteins - by designing protein sequences of a **Cys2-His2** zinc finger class based on a nucleic acid base triplet in a target nucleic acid sequence
INVENTOR: Choo Y; Isalan M; Klug A
PATENT ASSIGNEE: (MEDI-N)MEDICAL RES COUNCIL.
PATENT INFO: WO 9853059 A1 19981126 62p
APPLICATION INFO: WO 1998-GB1514 19980526
PRIORITY INFO: GB 1997-10807 19970523
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: 1999-045308 [04]
DESCRIPTION: Finger 3 of a NABP specific for a G12V mutant ras oncogene.

L6 ANSWER 34 OF 38 DGENE COPYRIGHT 2003 THOMSON DERWENT on STN
TI Preparation of nucleic acid binding proteins - by designing protein sequences of a **Cys2-His2** zinc finger class based on a nucleic acid base triplet in a target nucleic acid sequence
AN AAW84305 Peptide DGENE
AB The present sequence represents finger 1 of a **nucleic acid binding protein** (NABP) specific for a G12V mutant ras oncogene. The specification describes a method for preparing a NABP of the **Cys2-His2** zinc finger class capable of binding to a nucleic acid base triplet in a target nucleic

acid sequence. Binding to the 5' base of the triplet by an alpha-helical zinc finger nucleic acid binding motif in the protein is determined as follows: (a) if the 5' base in the triplet is A, then position +6 in the alpha -helix is Glu, Asn or Val; (b) if the 5' base in the triplet is C, then position +6 in the alpha-helix is Ser, Thr, Val, Ala, Glu or Asn. The methods can be used for designing a protein which is capable of binding to any predefined nucleic acid sequence. The NABPs can be used for the detection of target nucleic acid molecules. They can also be used in gene therapy, e.g. for the delivery of functional genes into defective genes, or the delivery of nonsense nucleic acid to disrupt undesired nucleic acid.

ACCESSION NUMBER: AAW84305 Peptide DGENE
TITLE: Preparation of nucleic acid binding proteins - by designing protein sequences of a **Cys2-His2** zinc finger class based on a nucleic acid base triplet in a target nucleic acid sequence
INVENTOR: Choo Y; Isalan M; Klug A
PATENT ASSIGNEE: (MEDI-N)MEDICAL RES COUNCIL.
PATENT INFO: WO 9853059 A1 19981126 62p
APPLICATION INFO: WO 1998-GB1514 19980526
PRIORITY INFO: GB 1997-10807 19970523
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: 1999-045308 [04]
DESCRIPTION: Finger 2 of a NABP specific for a G12V mutant ras oncogene.

L6 ANSWER 35 OF 38 DGENE COPYRIGHT 2003 THOMSON DERWENT on STN
TI Preparation of nucleic acid binding proteins - by designing protein sequences of a **Cys2-His2** zinc finger class based on a nucleic acid base triplet in a target nucleic acid sequence
AN AAW84304 Peptide DGENE
AB The present sequence represents finger 1 of a **nucleic acid binding protein** (NABP) specific for a G12V mutant ras oncogene. The specification describes a method for preparing a NABP of the **Cys2-His2** zinc finger class capable of binding to a nucleic acid base triplet in a target nucleic acid sequence. Binding to the 5' base of the triplet by an alpha-helical zinc finger nucleic acid binding motif in the protein is determined as follows: (a) if the 5' base in the triplet is A, then position +6 in the alpha -helix is Glu, Asn or Val; (b) if the 5' base in the triplet is C, then position +6 in the alpha-helix is Ser, Thr, Val, Ala, Glu or Asn. The methods can be used for designing a protein which is capable of binding to any predefined nucleic acid sequence. The NABPs can be used for the detection of target nucleic acid molecules. They can also be used in gene therapy, e.g. for the delivery of functional genes into defective genes, or the delivery of nonsense nucleic acid to disrupt undesired nucleic acid.

ACCESSION NUMBER: AAW84304 Peptide DGENE
TITLE: Preparation of nucleic acid binding proteins - by designing protein sequences of a **Cys2-His2** zinc finger class based on a nucleic acid base triplet in a target nucleic acid sequence
INVENTOR: Choo Y; Isalan M; Klug A
PATENT ASSIGNEE: (MEDI-N)MEDICAL RES COUNCIL.
PATENT INFO: WO 9853059 A1 19981126 62p
APPLICATION INFO: WO 1998-GB1514 19980526
PRIORITY INFO: GB 1997-10807 19970523
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: 1999-045308 [04]
DESCRIPTION: Finger 1 of a NABP specific for a G12V mutant ras oncogene.

L6 ANSWER 36 OF 38 DGENE COPYRIGHT 2003 THOMSON DERWENT on STN
TI Preparing **Cys2-His2** zinc finger class nucleic acid

binding proteins - capable of binding to a nucleic acid quadruplet, by mutating sites in model zinc finger domains according to a defined set of substitutions

AN AAV83634 DNA DGENE

AB The present sequence encodes a **nucleic acid binding protein** (NBP) of the **Cys2-His2** zinc finger class. The NBP is capable of binding to a nucleic acid quadruplet in a target sequence. The specification describers a method of preparing the NBP. The method is used for designing nucleic acid binding proteins which are useful in medicine. The proteins are specifically engineered to recognise particular nucleic acid sequences and as such are suitable for diagnosis of genetic disorders. The proteins can be used in the manufacture of chimeric restriction enzymes, in which a nucleic acid cleaving domain is fused to a nucleic acid binding domain comprising a zinc finger. Fusion protein comprising NBP and an integrase, e.g. viral integrase, can be used to target nucleic acid sequences in vivo. In gene therapy applications, the method may be targeted to the delivery of functional genes into defective genes, or the delivery of nonsense nucleic acid in order to disrupt undesired nucleic acid. Genes may also be delivered to known, repetitive stretches of nucleic acid, e.g. centromeres, together with an activating sequence such as an LCR. NBP can be specifically used to knockout cells having mutant proteins e.g. mutant ras. They can also be used to modulate the action of transcription factors, e.g. the activity of HIV tat may be reduced by NBP specific for HIV TAR. NBP may also be coupled to toxic molecules, e.g. nucleases, which are capable of selectively destroying cells which comprise a mutation in their endogenous nucleic acid.

ACCESSION NUMBER: AAV83634 DNA DGENE

TITLE: Preparing **Cys2-His2** zinc finger class nucleic acid binding proteins - capable of binding to a nucleic acid quadruplet, by mutating sites in model zinc finger domains according to a defined set of substitutions

INVENTOR: Choo Y; Isalan M; Klug A

PATENT ASSIGNEE: (MEDI-N)MEDICAL RES COUNCIL.

PATENT INFO: WO 9853058 A1 19981126 64p

APPLICATION INFO: WO 1998-GB1512 19980526

PRIORITY INFO: GB 1997-10809 19970523

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: 1999-024578 [02]

CROSS REFERENCES: P-PSDB: AAW87557

DESCRIPTION: DNA encoding a **nucleic acid binding protein**.

L6 ANSWER 37 OF 38 DGENE COPYRIGHT 2003 THOMSON DERWENT on STN

TI Preparation of nucleic acid binding proteins - by designing protein sequences of a **Cys2-His2** zinc finger class based on a nucleic acid base triplet in a target nucleic acid sequence

AN AAV99467 DNA DGENE

AB The present sequence encodes an anti-HIV zinc finger. The zinc finger was made to exemplify the invention. THe specification describes a method for preparing a **nucleic acid binding protein** (NABP) of the **Cys2-His2** zinc finger class capable of binding to a nucleic acid base triplet in a target nucleic acid sequence. Binding to the 5' base of the triplet by an alpha-helical zinc finger nucleic acid binding motif in the protein is determined as follows: (a) if the 5' base in the triplet is A, then position +6 in the alpha -helix is Glu, Asn or Val; (b) if the 5' base in the triplet is C, then position +6 in the alpha -helix is Ser, Thr, Val, Ala, Glu or Asn. The methods can be used for designing a protein which is capable of binding to any predefined nucleic acid sequence. The NABPs can be used for the detection of target nucleic acid molecules. They can also be used in gene therapy, e.g. for the delivery of functional genes into defective genes, or the delivery of nonsense nucleic acid to disrupt

undesired nucleic acid.

ACCESSION NUMBER: AAV99467 DNA DGENE
TITLE: Preparation of nucleic acid binding proteins - by designing protein sequences of a **Cys2-His2** zinc finger class based on a nucleic acid base triplet in a target nucleic acid sequence
INVENTOR: Choo Y; Isalan M; Klug A
PATENT ASSIGNEE: (MEDI-N)MEDICAL RES COUNCIL.
PATENT INFO: WO 9853059 A1 19981126 62p
APPLICATION INFO: WO 1998-GB1514 19980526
PRIORITY INFO: GB 1997-10807 19970523
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: 1999-045308 [04]
CROSS REFERENCES: P-PSDB: AAW84299
DESCRIPTION: DNA encoding an anti-HIV zinc finger.

L6 ANSWER 38 OF 38 EMBASE COPYRIGHT 2003 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
TI brlA requires both zinc fingers to induce development.
AB Expression of the Aspergillus nidulans brlA gene induces a developmental pathway leading to the production of asexual spores. We have introduced mutations into brlA that are expected to disrupt either or both **Cys2-His2** Zn(II) coordination sites postulated to exist in the BrlA polypeptide. The resultant brlA alleles fail to induce either the asexual reproductive pathway or the expression of development-specific genes. These data support the hypothesis that brlA encodes a **nucleic acid-binding protein** whose activity requires each of two zinc fingers.

ACCESSION NUMBER: 90105404 EMBASE
DOCUMENT NUMBER: 1990105404
TITLE: brlA requires both zinc fingers to induce development.
AUTHOR: Adams T.H.; Deising H.; Timberlake W.E.
CORPORATE SOURCE: Department of Genetics, University of Georgia,Athens, GA 30602, United States
SOURCE: Molecular and Cellular Biology, (1990) 10/4 (1815-1817). ISSN: 0270-7306 CODEN: MCEBD4
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English

L3 790 L2 AND METHOD

=> s l3 and production
L4 329 L3 AND PRODUCTION

=> s l4 and l1
L5 84 L4 AND L1

=> d l5 ti abs ibib 1-10

L5 ANSWER 1 OF 84 USPATFULL
TI Isolation and use of fetal urogenital sinus expressed sequences
AB The invention comprises methods for identifying biomarkers useful for prognostic or diagnostic assays of human prostate disease, and for identifying those fetal genes which are differentially expressed between prostate cancers versus normal or benign prostate.

ACCESSION NUMBER: 2002:279688 USPATFULL
TITLE: Isolation and use of fetal urogenital sinus expressed sequences
INVENTOR(S): Sikes, Robert A., Gordonsville, VA, UNITED STATES
Chung, Leland W.K., Lovingston, VA, UNITED STATES
Kim, Jin Hee, Santa Monica, CA, UNITED STATES
Fasciana, Claudia, Rotterdam, NETHERLANDS
Trapman, Jan, Mijnsheerenland, NETHERLANDS

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2002155119	A1	20021024
APPLICATION INFO.:	US 2001-933797	A1	20010822 (9)

RELATED APPLN. INFO.: Continuation of Ser. No. US 2000-482933, filed on 14 Jan 2000, ABANDONED Continuation of Ser. No. WO 1999-US10746, filed on 14 May 1999, UNKNOWN

	NUMBER	DATE
PRIORITY INFORMATION:	US 1998-85383P	19980514 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: PENNIE & EDMONDS LLP, 1667 K STREET NW, SUITE 1000, WASHINGTON, DC, 20006
NUMBER OF CLAIMS: 43
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 472 Drawing Page(s)
LINE COUNT: 13107

L5 ANSWER 2 OF 84 USPATFULL
TI Compositions and methods for ovarian cancer therapy and diagnosis
AB Compositions and methods for the therapy and diagnosis of cancer, such as ovarian cancer, are disclosed. Compositions may comprise one or more ovarian carcinoma proteins, immunogenic portions thereof, polynucleotides that encode such portions or antibodies or immune system cells specific for such proteins. Such compositions may be used, for example, for the prevention and treatment of diseases such as ovarian cancer. Methods are further provided for identifying tumor antigens that are secreted from ovarian carcinomas and/or other tumors. Polypeptides and polynucleotides as provided herein may further be used for the diagnosis and monitoring of ovarian cancer.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2002:275909 USPATFULL
TITLE: Compositions and methods for ovarian cancer therapy and

diagnosis

INVENTOR(S):	Benson, Darin R., Seattle, WA, United States Lodes, Michael J., Seattle, WA, United States Mitcham, Jennifer L., Redmond, WA, United States King, Gordon E., Seattle, WA, United States
PATENT ASSIGNEE(S):	Corixa Corporation, Seattle, WA, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6468758	B1	20021022
APPLICATION INFO.:	US 1999-397787		19990916 (9)

RELATED APPLN. INFO.:	Continuation-in-part of Ser. No. US 1999-246429, filed on 8 Feb 1999 Continuation-in-part of Ser. No. US 1998-159320, filed on 23 Sep 1998, now abandoned
DOCUMENT TYPE:	Utility
FILE SEGMENT:	GRANTED
PRIMARY EXAMINER:	Brusca, John S.
ASSISTANT EXAMINER:	Moran, Margorie A.
LEGAL REPRESENTATIVE:	Seed Intellectual Property Law Group PLLC
NUMBER OF CLAIMS:	3
EXEMPLARY CLAIM:	1
NUMBER OF DRAWINGS:	32 Drawing Figure(s); 32 Drawing Page(s)
LINE COUNT:	5338

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 3 OF 84 USPATFULL

TI Nucleic acids, proteins and antibodies

AB This invention relates to newly identified prostate or prostate cancer related polynucleotides, the polypeptides encoded by these polynucleotides herein collectively referred to as "prostate cancer antigens," and to the complete gene sequences associated therewith and to the expression products thereof, and to antibodies that immunospecifically bind these polypeptides, as well as the use of such prostate cancer polynucleotides, antigens, and antibodies for detection, prevention, prognosis, and treatment of disorders of the reproductive system, particularly disorders of the prostate, including, but not limited to, the presence of prostate cancer and prostate cancer metastases. More specifically, isolated prostate cancer nucleic acid molecules are provided encoding novel prostate cancer polypeptides. Novel prostate cancer polypeptides and antibodies that bind to these polypeptides are provided. Also provided are vectors, host cells, and recombinant and synthetic methods for producing human prostate cancer polynucleotides, polypeptides, and/or antibodies. The invention further relates to diagnostic and therapeutic methods useful for diagnosing, treating, preventing and/or prognosing disorders related to the prostate, including prostate cancer, and therapeutic methods for treating such disorders. The invention further relates to screening methods for identifying agonists and antagonists of polynucleotides and polypeptides of the invention. The invention further relates to methods and/or compositions for inhibiting or promoting the **production** and/or function of the polypeptides of the invention.

ACCESSION NUMBER:	2002:273550 USPATFULL
TITLE:	Nucleic acids, proteins and antibodies
INVENTOR(S):	Rosen, Craig A., Laytonsville, MD, UNITED STATES Ruben, Steven M., Olney, MD, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2002151681	A1	20021017
APPLICATION INFO.:	US 2001-925300	A1	20010810 (9)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. WO 2000-US5988, filed on 8 Mar 2000, UNKNOWN

	NUMBER	DATE
PRIORITY INFORMATION:	US 1999-124270P	19990312 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: HUMAN GENOME SCIENCES INC, 9410 KEY WEST AVENUE, ROCKVILLE, MD, 20850
NUMBER OF CLAIMS: 23
EXEMPLARY CLAIM: 1
LINE COUNT: 29771

L5 ANSWER 4 OF 84 USPATFULL
TI PEI: DNA vector formulations for in vitro and in vivo gene delivery
AB The present invention relates generally to the fields of nucleic acid transfection. More particularly, it concerns novel polycation:nucleic acid compositions, methods of preparation of such compositions and methods of transfecting cells with such compositions.

ACCESSION NUMBER: 2002:272939 USPATFULL
TITLE: PEI: DNA vector formulations for in vitro and in vivo gene delivery
INVENTOR(S): Cristiano, Richard J., Pearland, TX, UNITED STATES
Yamashita, Motoyuki, Kochi City, JAPAN
PATENT ASSIGNEE(S): Board of Regents, The University of Texas System (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2002151060	A1	20021017
APPLICATION INFO.:	US 2001-962922	A1	20010925 (9)

	NUMBER	DATE
PRIORITY INFORMATION:	US 2000-235237P	20000925 (60)
	US 2000-235635P	20000926 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: FULBRIGHT & JAWORSKI L.L.P., A REGISTERED LIMITED LIABILITY PARTNERSHIP, SUITE 2400, 600 CONGRESS AVENUE, AUSTIN, TX, 78701
NUMBER OF CLAIMS: 141
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 31 Drawing Page(s)
LINE COUNT: 7002

L5 ANSWER 5 OF 84 USPATFULL
TI End selection in directed evolution
AB This invention provides methods of obtaining novel polynucleotides and encoded polypeptides by the use of non-stochastic methods of directed evolution (DirectEvolution.TM.). A particular advantage of end-selection-based methods is the ability to recover full-length polynucleotides from a library of progeny molecules generated by mutagenesis methods. These methods include non-stochastic polynucleotide site-saturation mutagenesis (Gene Site Saturation Mutagenesis.TM.) and non-stochastic polynucleotide reassembly (GeneReassembly.TM.). This invention provides methods of obtaining novel enzymes that have optimized physical &/or biological properties. Through use of the claimed methods, genetic vaccines, enzymes, small molecules, and other desirable molecules can be evolved towards desirable properties. For

example, vaccine vectors, can be obtained that exhibit increased efficacy for use as genetic vaccines. Vectors obtained by using the methods can have, for example, enhanced antigen expression, increased uptake into a cell, increased stability in a cell, ability to tailor an immune response, and the like. Furthermore, this invention provides methods of obtaining a variety of novel biologically active molecules, in the fields of antibiotics, pharmacotherapeutics, and transgenic traits.

ACCESSION NUMBER: 2002:265886 USPATFULL
TITLE: End selection in directed evolution
INVENTOR(S): Short, Jay M., Rancho Santa Fe, CA, UNITED STATES
Frey, Gerhard Johann, San Diego, CA, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2002146762	A1	20021010
APPLICATION INFO.:	US 2001-885551	A1	20010619 (9)

RELATED APPLN. INFO.: Continuation of Ser. No. US 2000-522289, filed on 9 Mar 2000, PATENTED Continuation-in-part of Ser. No. US 2000-498557, filed on 4 Feb 2000, PENDING Continuation-in-part of Ser. No. US 2000-495052, filed on 31 Jan 2000, PENDING Continuation-in-part of Ser. No. US 1999-332835, filed on 14 Jun 1999, ABANDONED Continuation-in-part of Ser. No. US 1999-276860, filed on 26 Mar 1999, PATENTED Continuation-in-part of Ser. No. US 1999-267118, filed on 9 Mar 1999, PATENTED Continuation-in-part of Ser. No. US 1999-246178, filed on 4 Feb 1999, PATENTED Continuation-in-part of Ser. No. US 1998-185373, filed on 3 Nov 1998, PATENTED Continuation of Ser. No. US 1996-760489, filed on 5 Dec 1996, PATENTED

	NUMBER	DATE
PRIORITY INFORMATION:	US 1995-8311P	19951207 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: GARY CARY WARE & FRIENDENRICH LLP, 4365 EXECUTIVE DRIVE, SUITE 1600, SAN DIEGO, CA, 92121-2189
NUMBER OF CLAIMS: 4
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 7 Drawing Page(s)
LINE COUNT: 8987

L5 ANSWER 6 OF 84 USPATFULL
TI Exonuclease-mediated gene assembly in directed evolution
AB A directed evolution process comprising novel methods for generating improved progeny molecules having desirable properties, including, for example, a **method** for rapid and facilitated **production** from a parental polynucleotide template, of a set of mutagenized progeny polynucleotides wherein at least one codon encoding each of the 20 naturally encoded amino acids is represented at each original codon position. This **method**, termed site-saturation mutagenesis, or simply saturation mutagenesis, is preferably based on the use of the degenerate N,N,G/T sequence. Also, a **method** of producing from a parental polypeptide template, a set of mutagenized progeny polypeptides wherein each of the 20 naturally encoded amino acids is represented at each original amino acid position. Also, other mutagenization processes that can be used in combination with, or in lieu of, saturation mutagenesis, including, for example: (a) assembly and/or reassembly of polynucloetide building blocks (including sections

of genes &/or of gene families) mediated by a source of exonuclease activity such as exonuclease III; and (b) introduction of two or more related polynucleotides into a suitable host cell such that a hybrid polynucleotide is generated by recombination and reductive reassortment. Also molecular property screening methods, including a preferred **method**, termed end selection, comprised of using an enzyme, such as a topoisomerase, a restriction endonuclease, &/or a nicking enzyme (such as N. BstNB I), to detect a specific terminal sequence in a working polynucleotide, to produce a ligatable end thereat, and to ligate and clone the working polynucleotide.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	2002:258824 USPATFULL		
TITLE:	Exonuclease-mediated gene assembly in directed evolution		
INVENTOR(S):	Short, Jay M., Rancho Santa Fe, CA, UNITED STATES		
PATENT ASSIGNEE(S):	Diversa Corporation (U.S. corporation)		
	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2002142394	A1	20021003
APPLICATION INFO.:	US 2002-87426	A1	20020301 (10)
RELATED APPLN. INFO.:	Continuation of Ser. No. US 1999-276860, filed on 26 Mar 1999, GRANTED, Pat. No. US 6352842 Continuation-in-part of Ser. No. US 1999-267118, filed on 9 Mar 1999, GRANTED, Pat. No. US 6238884 Continuation-in-part of Ser. No. US 1999-246178, filed on 4 Feb 1999, GRANTED, Pat. No. US 6171820 Continuation-in-part of Ser. No. US 1998-185373, filed on 3 Nov 1998, GRANTED, Pat. No. US 6335179 Continuation of Ser. No. US 1996-760489, filed on 5 Dec 1996, GRANTED, Pat. No. US 5830696 Continuation-in-part of Ser. No. US 1996-677112, filed on 9 Jul 1996, GRANTED, Pat. No. US 5965408		
	NUMBER	DATE	
PRIORITY INFORMATION:	US 1995-8311P	19951207 (60)	
	US 1995-8316P	19951207 (60)	
DOCUMENT TYPE:	Utility		
FILE SEGMENT:	APPLICATION		
LEGAL REPRESENTATIVE:	HALE AND DORR LLP, 300 PARK AVENUE, NEW YORK, NY, 10022		
NUMBER OF CLAIMS:	1		
EXEMPLARY CLAIM:	1		
NUMBER OF DRAWINGS:	1 Drawing Page(s)		
LINE COUNT:	4637		

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 7 OF 84 USPATFULL

TI Detection of nucleic acids by multiple sequential invasive cleavages 02

AB The present invention relates to means for the detection and characterization of nucleic acid sequences, as well as variations in nucleic acid sequences. The present invention also relates to methods for forming a nucleic acid cleavage structure on a target sequence and cleaving the nucleic acid cleavage structure in a site-specific manner. The structure-specific nuclease activity of a variety of enzymes is used to cleave the target-dependent cleavage structure, thereby indicating the presence of specific nucleic acid sequences or specific variations thereof. The present invention further relates to methods and devices for the separation of nucleic acid molecules based on charge. The present invention also provides methods for the detection of non-target cleavage products via the formation of a complete and activated protein

binding region. The invention further provides sensitive and specific methods for the detection of human cytomegalovirus nucleic acid in a sample.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	2002:254176 USPATFULL
TITLE:	Detection of nucleic acids by multiple sequential invasive cleavages 02
INVENTOR(S):	Hall, Jeff G., Madison, WI, United States Lyamichev, Victor I., Madison, WI, United States Mast, Andrea L., Madison, WI, United States Brow, Mary Ann D., Madison, WI, United States
PATENT ASSIGNEE(S):	Third Wave Technologies, Inc, Madison, WI, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6458535	B1	20021001
APPLICATION INFO.:	US 1999-350597		19990709 (9)
RELATED APPLN. INFO.:	Continuation of Ser. No. US 1997-823516, filed on 24 Mar 1997, now patented, Pat. No. US 5994069 Continuation-in-part of Ser. No. US 1996-759038, filed on 2 Dec 1996, now patented, Pat. No. US 6090543 Continuation-in-part of Ser. No. US 1996-756386, filed on 26 Nov 1996, now patented, Pat. No. US 5085557 Continuation-in-part of Ser. No. US 1996-682853, filed on 12 Jul 1996, now patented, Pat. No. US 6001567 Continuation-in-part of Ser. No. US 1996-599491, filed on 24 Jan 1996, now patented, Pat. No. US 5846717, issued on 8 Dec 1998		
DOCUMENT TYPE:	Utility		
FILE SEGMENT:	GRANTED		
PRIMARY EXAMINER:	Jones, W. Gary		
ASSISTANT EXAMINER:	Souaya, Jehanne		
LEGAL REPRESENTATIVE:	Medlen & Carroll, LLP		
NUMBER OF CLAIMS:	27		
EXEMPLARY CLAIM:	1		
NUMBER OF DRAWINGS:	170 Drawing Figure(s); 128 Drawing Page(s)		
LINE COUNT:	13831		

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 8 OF 84 USPATFULL

TI Compositions and methods for the therapy and diagnosis of ovarian cancer

AB Compositions and methods for the therapy and diagnosis of cancer, particularly ovarian cancer, are disclosed. Illustrative compositions comprise one or more ovarian tumor polypeptides, immunogenic portions thereof, polynucleotides that encode such polypeptides, antigen presenting cell that expresses such polypeptides, and T cells that are specific for cells expressing such polypeptides. The disclosed compositions are useful, for example, in the diagnosis, prevention and/or treatment of diseases, particularly ovarian cancer.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	2002:243051 USPATFULL
TITLE:	Compositions and methods for the therapy and diagnosis of ovarian cancer
INVENTOR(S):	Algate, Paul A., Issaquah, WA, UNITED STATES Jones, Robert, Seattle, WA, UNITED STATES Harlocker, Susan L., Seattle, WA, UNITED STATES
PATENT ASSIGNEE(S):	Corixa Corporation, Seattle, WA, UNITED STATES, 98104 (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2002132237	A1	20020919
APPLICATION INFO.:	US 2001-867701	A1	20010529 (9)

	NUMBER	DATE
PRIORITY INFORMATION:	US 2000-207484P	20000526 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: SEED INTELLECTUAL PROPERTY LAW GROUP PLLC, 701 FIFTH AVE, SUITE 6300, SEATTLE, WA, 98104-7092
NUMBER OF CLAIMS: 11
EXEMPLARY CLAIM: 1
LINE COUNT: 25718
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 9 OF 84 USPATFULL
TI Methods using genetic package display for selecting internalizing ligands for gene delivery
AB A genetic package display system is presented for selecting internalizing ligands for gene delivery. The genetic package carries a reporter, selectable marker, or a specifically detectable nucleic acid sequence and presents a ligand on its surface. More specifically, a library of potential ligands may be screened for the ability to successfully transduce target cells.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2002:238816 USPATFULL
TITLE: Methods using genetic package display for selecting internalizing ligands for gene delivery
INVENTOR(S): Larocca, David, Encinitas, CA, United States
Baird, Andrew, San Diego, CA, United States
Kassner, Paul, Hayward, CA, United States
PATENT ASSIGNEE(S): Selective Genetics, Inc., San Diego, CA, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6451527	B1	20020917
APPLICATION INFO.:	US 1999-258689		19990226 (9)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 1998-193445, filed on 17 Nov 1998 Continuation-in-part of Ser. No. US 1998-195379, filed on 17 Nov 1998 Continuation-in-part of Ser. No. US 1998-141631, filed on 28 Aug 1998, now abandoned

	NUMBER	DATE
PRIORITY INFORMATION:	US 1997-57067P	19970829 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: GRANTED
PRIMARY EXAMINER: Ponnaluri, Padmashri
LEGAL REPRESENTATIVE: Seed Intellectual Property Law Group PLLC
NUMBER OF CLAIMS: 10
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 17 Drawing Figure(s); 13 Drawing Page(s)
LINE COUNT: 2048
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 10 OF 84 USPATFULL
TI Nod2 nucleic acids and proteins

AB The present invention relates to intracellular signalling molecules, in particular the Nod2 protein and nucleic acids encoding the Nod2 protein. The present invention provides isolated nucleotide sequence encoding Nod2, isolated Nod2 peptides, antibodies that specifically bind Nod2, methods for the detection of Nod2, and methods for screening compounds for the ability to alter Nod2 associated signal transduction.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	2002:235484 USPATFULL		
TITLE:	Nod2 nucleic acids and proteins		
INVENTOR(S):	Nunez, Gabriel, Ann Arbor, MI, UNITED STATES Inohara, Naohiro, Ann Arbor, MI, UNITED STATES Ogura, Yasunori, Ann Arbor, MI, UNITED STATES		
	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2002127673	A1	20020912
APPLICATION INFO.:	US 2001-14269	A1	20011026 (10)
	NUMBER		DATE
PRIORITY INFORMATION:	US 2000-244289P		20001030 (60)
DOCUMENT TYPE:	Utility		
FILE SEGMENT:	APPLICATION		
LEGAL REPRESENTATIVE:	David A. Casimir, MEDLEN & CARROLL, LLP, Suite 350, 101 Howard Street, San Francisco, CA, 94105		
NUMBER OF CLAIMS:	26		
EXEMPLARY CLAIM:	1		
NUMBER OF DRAWINGS:	21 Drawing Page(s)		
LINE COUNT:	5519		

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

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L5 ANSWER 11 OF 84 USPATFULL
TI Methods for generating polynucleotides having desired characteristics by iterative selection and recombination
AB A **method** for DNA reassembly after random fragmentation, and its application to mutagenesis of nucleic acid sequences by in vitro or in vivo recombination is described. In particular, a **method** for the **production** of nucleic acid fragments or polynucleotides encoding mutant proteins is described. The present invention also relates to a **method** of repeated cycles of mutagenesis, shuffling and selection which allow for the directed molecular evolution in vitro or in vivo of proteins.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	2002:224459 USPATFULL
TITLE:	Methods for generating polynucleotides having desired characteristics by iterative selection and recombination
INVENTOR(S):	Stemmer, Willem P. C., Los Gatos, CA, United States Crameri, Andreas, Mountain View, CA, United States
PATENT ASSIGNEE(S):	Maxygen, Inc., Redwood City, CA, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6444468	B1	20020903
APPLICATION INFO.:	US 2000-724958		20001128 (9)
RELATED APPLN. INFO.:	Continuation of Ser. No. US 1998-133508, filed on 12 Aug 1998, now patented, Pat. No. US 6287861 Continuation of Ser. No. US 537874, now patented, Pat. No. US 5830721 Continuation-in-part of Ser. No. US 1994-198431, filed on 17 Feb 1994, now patented, Pat. No. US 5605793		
DOCUMENT TYPE:	Utility		
FILE SEGMENT:	GRANTED		
PRIMARY EXAMINER:	Whisenant, Ethan C.		
LEGAL REPRESENTATIVE:	Kruse, Norman, Liebeschuetz, Joe		
NUMBER OF CLAIMS:	62		
EXEMPLARY CLAIM:	1		
NUMBER OF DRAWINGS:	16 Drawing Figure(s); 15 Drawing Page(s)		
LINE COUNT:	4266		

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 12 OF 84 USPATFULL
TI End selection in directed evolution
AB A directed evolution process comprising novel methods for generating improved progeny molecules having desirable properties, including, for example, a **method** for rapid and facilitated **production** from a parental polynucleotide template, of a set of mutagenized progeny polynucleotides wherein at least one codon encoding each of the 20 naturally encoded amino acids is represented at each original codon position. This **method**, termed site-saturation mutagenesis, or simply saturation mutagenesis, is preferably based on the use of the degenerate N,N,G/T sequence. Also, a **method** of producing from a parental polypeptide template, a set of mutagenized progeny polypeptides wherein each of the 20 naturally encoded amino acids is represented at each original amino acid position. Also, other mutagenization processes that can be used in combination with, or in lieu of, saturation mutagenesis, including, for example: (a) assembly and/or reassembly of polynucloetide building blocks, which building blocks can be sections of genes &/or of gene families; and (b) introduction of two or more related polynucleotides into a suitable host cell such that a hybrid polynucleotide is generated by recombination and

reductive reassortment. Also, vector and expression vehicles including such polynucleotides and correspondingly expressed polypeptides. Also molecular property screening methods, including a preferred **method**, termed end selection, comprised of using an enzyme, such as a topoisomerase, a restriction endonuclease, &/or a nicking enzyme (such as N. BstNB I), to detect a specific terminal sequence in a working polynucleotide, to produce a ligatable end thereat, and to ligate and clone the working polynucleotide.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	2002:221318 USPATFULL
TITLE:	End selection in directed evolution
INVENTOR(S):	Short, Jay M., Rancho Santa Fe, CA, UNITED STATES Frey, Gerhard Johann, San Diego, CA, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2002119457	A1	20020829
APPLICATION INFO.:	US 2001-867262	A1	20010529 (9)

RELATED APPLN. INFO.: Continuation of Ser. No. US 1999-267118, filed on 9 Mar 1999, PATENTED Continuation-in-part of Ser. No. US 1999-246178, filed on 4 Feb 1999, PATENTED Continuation-in-part of Ser. No. US 1998-185373, filed on 3 Nov 1998, PATENTED Continuation-in-part of Ser. No. US 1996-760489, filed on 5 Dec 1996, PATENTED

	NUMBER	DATE
PRIORITY INFORMATION:	US 1995-8311P	19951207 (60)

DOCUMENT TYPE:	Utility
FILE SEGMENT:	APPLICATION
LEGAL REPRESENTATIVE:	GARY CARY WARE & FRIENDENRICH LLP, 4365 EXECUTIVE DRIVE, SUITE 1600, SAN DIEGO, CA, 92121-2189
NUMBER OF CLAIMS:	12
EXEMPLARY CLAIM:	1
NUMBER OF DRAWINGS:	5 Drawing Page(s)
LINE COUNT:	4507

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 13 OF 84 USPATFULL

TI **Method** of DNA shuffling with polynucleotides produced by blocking or interrupting a synthesis or amplification process

AB Disclosed is a process of performing Sexual PCR which includes generating random polynucleotides by interrupting or blocking synthesis or amplification process to slow or halt synthesis or amplification of at least one polynucleotides, optionally amplifying the polynucleotides, and reannealing the polynucleotides to produce random mutant polynucleotides. Also provided are vector and expression vehicles including such mutant polynucleotides, polypeptides expressed by the mutant polynucleotides and a **method** for producing random polypeptides.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	2002:217027 USPATFULL
TITLE:	**Method** of DNA shuffling with polynucleotides produced by blocking or interrupting a synthesis or amplification process
INVENTOR(S):	Short, Jay M., Encinitas, CA, United States
PATENT ASSIGNEE(S):	Diversa Corporation, San Diego, CA, United States (U.S. corporation)

	NUMBER	KIND	DATE

PATENT INFORMATION:	US 6440668	B1	20020827
APPLICATION INFO.:	US 1999-376727		19990817 (9)
RELATED APPLN. INFO.:	Continuation of Ser. No. US 1996-677112, filed on 9 Jul 1996, now patented, Pat. No. US 5965408		
DOCUMENT TYPE:	Utility		
FILE SEGMENT:	GRANTED		
PRIMARY EXAMINER:	Zitomer, Stephanie		
LEGAL REPRESENTATIVE:	Gray Cary Ware & Freidenrich LLP, Haile, Lisa A.		
NUMBER OF CLAIMS:	12		
EXEMPLARY CLAIM:	1		
NUMBER OF DRAWINGS:	6 Drawing Figure(s); 6 Drawing Page(s)		
LINE COUNT:	2614		

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 14 OF 84 USPATFULL

TI Systematic evolution of ligands by exponential enrichment: photoselection of nucleic acid ligands and solution selex

AB A **method** for identifying nucleic acid ligands to target molecules using the SELEX procedure. Nucleic acid candidate sequences contain photoreactive groups. After exposure of the nucleic acid sequences to the target molecule, nucleic acid-target molecule complexes are formed between nucleic acids having increased affinity to the target molecule and the target molecule. The complexes are irradiated such that photocrosslinks form between the photoreactive groups of the bound nucleic acids and the target molecule. The photocrosslinked complexes are separated from unbound nucleic acids, and the nucleic acids amplified to yield a ligand-enriched mixture of nucleic acids.

Described herein are methods for improved partitioning between high and low affinity nucleic acid ligands identified through the SELEX **method**, termed solution SELEX. The solution SELEX **method** achieves partitioning between high and low affinity nucleic acid-target complexes through a number of methods, including (1) primer extension inhibition which results in differentiable cDNA products. Primer extension inhibition is achieved with the use of nucleic acid polymerases, including DNA or RNA polymerases, reverse transcriptase, and Qβ-replicase; (2) exonuclease hydrolysis inhibition which results in only the highest affinity ligands amplifying during PCR. This is achieved with the use of any 3'→5' double-stranded exonuclease; (3) linear to circle formation to generate molecules amplifiable during PCR; or (4) PCR amplification of single-stranded nucleic acids. A central theme of the **method** of the present invention is that the nucleic acid candidate mixture is screened in solution and results in preferential amplification of the highest affinity RNA ligand or catalytic RNA.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	2002:198552 USPATFULL
TITLE:	Systematic evolution of ligands by exponential enrichment: photoselection of nucleic acid ligands and solution selex
INVENTOR(S):	Gold, Larry, Boulder, CO, UNITED STATES Willis, Michael, San Diego, CA, UNITED STATES Koch, Tad, Boulder, CO, UNITED STATES Ringquist, Steven, Oceanside, CA, UNITED STATES Jensen, Kirk, New York, NY, UNITED STATES Atkinson, Brent, Winterthur, SWITZERLAND
PATENT ASSIGNEE(S):	SomaLogic, Inc. (U.S. corporation)

	NUMBER	KIND	DATE

PATENT INFORMATION: US 2002106652 A1 20020808
APPLICATION INFO.: US 2001-882246 A1 20010614 (9)
RELATED APPLN. INFO.: Division of Ser. No. US 1999-459553, filed on 13 Dec 1999, PATENTED Division of Ser. No. US 1998-93293, filed on 8 Jun 1998, PATENTED Continuation of Ser. No. US 1996-612895, filed on 8 Mar 1996, PATENTED A 371 of International Ser. No. WO 1994-US10562, filed on 16 Sep 1994, UNKNOWN Continuation-in-part of Ser. No. US 1993-143564, filed on 25 Oct 1993, ABANDONED Continuation-in-part of Ser. No. US 1993-123935, filed on 17 Sep 1993, ABANDONED Continuation-in-part of Ser. No. US 1991-714131, filed on 10 Jun 1991, PATENTED Continuation-in-part of Ser. No. US 1990-536428, filed on 11 Jun 1990, ABANDONED Continuation-in-part of Ser. No. US 1992-931473, filed on 17 Aug 1992, PATENTED Division of Ser. No. US 1991-714131, filed on 10 Jun 1991, PATENTED
DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: Swanson & Bratschun, L.L.C., Suite 330, 1745 Shea Center Drive, Highlands Ranch, CO, 80129
NUMBER OF CLAIMS: 4
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 35 Drawing Page(s)
LINE COUNT: 2574
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 15 OF 84 USPATFULL
TI Methods for generating polynucleotides having desired characteristics by iterative selection and recombination
AB A **method** for DNA reassembly after random fragmentation, and its application to mutagenesis of nucleic acid sequences by in vitro or in vivo recombination is described. In particular, a **method** for the **production** of nucleic acid fragments or polynucleotides encoding mutant proteins is described. The present invention also relates to a **method** of repeated cycles of mutagenesis, shuffling and selection which allow for the directed molecular evolution in vitro or in vivo of proteins.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2002:174999 USPATFULL
TITLE: Methods for generating polynucleotides having desired characteristics by iterative selection and recombination
INVENTOR(S): Stemmer, Willem P.C., Los Gatos, CA, United States
PATENT ASSIGNEE(S): Maxygen, Inc., Redwood City, CA, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6420175	B1	20020716
APPLICATION INFO.:	US 1999-231253		19990115 (9)

RELATED APPLN. INFO.: Continuation of Ser. No. US 1998-100856, filed on 18 Jun 1998, now patented, Pat. No. US 6132970 Continuation of Ser. No. US 537874, now patented, Pat. No. US 5830721 Continuation-in-part of Ser. No. US 1994-198431, filed on 17 Feb 1994, now patented, Pat. No. US 5605793
DOCUMENT TYPE: Utility
FILE SEGMENT: GRANTED
PRIMARY EXAMINER: Whisenant, Ethan C.
LEGAL REPRESENTATIVE: Kruse, Norman, Liebeschuetz, Joe

NUMBER OF CLAIMS: 15
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 15 Drawing Figure(s); 15 Drawing Page(s)
LINE COUNT: 3737
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 16 OF 84 USPATFULL
TI Gene markers useful for detecting skin damage in response to ultraviolet radiation
AB The cellular response to ultraviolet radiation exposure has been characterized on the molecular level through the use of high density gene array technology. Nucleic acid molecules and protein molecules, the expression of which are repressed or induced in response to ultraviolet radiation exposure, are identified according to a temporal pattern of altered expression post ultraviolet radiation exposure. Methods are disclosed that utilized these ultraviolet radiation-regulated molecules as markers for ultraviolet radiation exposure. Other screening methods of the invention are designed for the identification of compounds that modulate the response of a cell to ultraviolet radiation exposure. The invention also provides compositions useful for drug screening or pharmaceutical purposes.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2002:171875 USPATFULL
TITLE: Gene markers useful for detecting skin damage in response to ultraviolet radiation
INVENTOR(S): Blumenberg, Miroslav, New York, NY, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2002090624	A1	20020711
APPLICATION INFO.:	US 2001-947870	A1	20010906 (9)

	NUMBER	DATE
PRIORITY INFORMATION:	US 2000-231454P	20000908 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: HALE AND DORR, LLP, 60 STATE STREET, BOSTON, MA, 02109
NUMBER OF CLAIMS: 97
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 6 Drawing Page(s)
LINE COUNT: 10110
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 17 OF 84 USPATFULL
TI Methods for generating polynucleotides having desired characteristics by iterative selection and recombination
AB A **method** for DNA reassembly after random fragmentation, and its application to mutagenesis of nucleic acid sequences by in vitro or in vivo recombination is described. In particular, a **method** for the **production** of nucleic acid fragments or polynucleotides encoding mutant proteins is described. The present invention also relates to a **method** of repeated cycles of mutagenesis, shuffling and selection which allow for the directed molecular evolution in vitro or in vivo of proteins.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2002:160573 USPATFULL
TITLE: Methods for generating polynucleotides having desired characteristics by iterative selection and recombination

INVENTOR(S): Stemmer, Willem P. C., Los Gatos, CA, United States
Cramieri, Andreas M., Mountain View, CA, United States
PATENT ASSIGNEE(S): Maxygen, Inc., Redwood City, CA, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6413774	B1	20020702
APPLICATION INFO.:	US 1999-240734		19990129 (9)

RELATED APPLN. INFO.: Continuation of Ser. No. US 1996-621859, filed on 25 Mar 1996, now patented, Pat. No. US 6117679 Continuation-in-part of Ser. No. US 1995-564955, filed on 30 Nov 1995 Continuation-in-part of Ser. No. WO 1995-US2126, filed on 17 Feb 1995, now patented, Pat. No. WO 5811238 Continuation of Ser. No. US 1994-198431, filed on 17 Feb 1994, now patented, Pat. No. US 5605793
DOCUMENT TYPE: Utility
FILE SEGMENT: GRANTED
PRIMARY EXAMINER: Whisenant, Ethan
LEGAL REPRESENTATIVE: Kruse, Norman J., Quine, Jonathan Alan, Law office of Jonathan Alan Quine
NUMBER OF CLAIMS: 36
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 35 Drawing Figure(s); 37 Drawing Page(s)
LINE COUNT: 6312
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 18 OF 84 USPATFULL
TI Compositions and methods for ovarian cancer therapy and diagnosis
AB Compositions and methods for the therapy and diagnosis of cancer, such as ovarian cancer, are disclosed. Compositions may comprise one or more ovarian carcinoma proteins, immunogenic portions thereof, polynucleotides that encode such portions or antibodies or immune system cells specific for such proteins. Such compositions may be used, for example, for the prevention and treatment of diseases such as ovarian cancer. Methods are further provided for identifying tumor antigens that are secreted from ovarian carcinomas and/or other tumors. Polypeptides and polynucleotides as provided herein may further be used for the diagnosis and monitoring of ovarian cancer.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2002:148574 USPATFULL
TITLE: Compositions and methods for ovarian cancer therapy and diagnosis
INVENTOR(S): Benson, Darin R., Seattle, WA, UNITED STATES
Lodes, Michael J., Seattle, WA, UNITED STATES
Mitcham, Jennifer L., Redmond, WA, UNITED STATES
King, Gordon E., Shoreline, WA, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2002076715	A1	20020620
APPLICATION INFO.:	US 2001-876889	A1	20010606 (9)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 1999-397787, filed on 16 Sep 1999, PENDING Continuation-in-part of Ser. No. US 1999-246429, filed on 8 Feb 1999, ABANDONED Continuation-in-part of Ser. No. US 1998-159320, filed on 23 Sep 1998, ABANDONED
DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: SEED INTELLECTUAL PROPERTY LAW GROUP PLLC, 701 FIFTH AVE, SUITE 6300, SEATTLE, WA, 98104-7092

NUMBER OF CLAIMS: 9
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 33 Drawing Page(s)
LINE COUNT: 7207
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 19 OF 84 USPATFULL
TI 98P7C3: homeodomain protein highly expressed in various cancers
AB A novel gene (designated 98P7C3) and its encoded protein are described. While 98P7C3 exhibits tissue-restricted expression in normal adult tissue, it is aberrantly expressed in multiple cancers including prostate, bladder, kidney, lung, breast, uterine, cervical, stomach, rectal and colon cancers. Consequently, 98P7C3 provides a diagnostic and/or therapeutic target for cancers, and the 98P7C3 gene or fragment thereof, or its encoded protein or a fragment thereof used to elicit an immune response.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2002:133493 USPATFULL
TITLE: 98P7C3: homeodomain protein highly expressed in various cancers
INVENTOR(S): Challita-Eid, Pia M., Encino, CA, UNITED STATES
Hubert, Rene S., Los Angeles, CA, UNITED STATES
Faris, Mary, Los Angeles, CA, UNITED STATES
Afar, Daniel E.H., Brisbane, CA, UNITED STATES
Levin, Elana, Los Angeles, CA, UNITED STATES
Mitchell, Steve Chappell, Santa Monica, CA, UNITED STATES
Jakobovits, Aya, Beverly Hills, CA, UNITED STATES

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2002068345	A1	20020606	
APPLICATION INFO.:	US 2001-866359	A1	20010524	(9)

	NUMBER	DATE
PRIORITY INFORMATION:	US 2000-207138P	20000524 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: GATES & COOPER LLP, HOWARD HUGHES CENTER, 6701 CENTER DRIVE WEST, SUITE 1050, LOS ANGELES, CA, 90045
NUMBER OF CLAIMS: 55
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 20 Drawing Page(s)
LINE COUNT: 6137
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 20 OF 84 USPATFULL
TI Methods using genetic package display for detecting and identifying protein-protein interactions that facilitate internalization and transgene expression and cells or tissues competent for the same and methods for evolving gene delivery vectors
AB A genetic package display system and methodology for probing protein-protein interactions that lead to cell transduction, selecting and/or identifying internalizing ligands, target cells and tissues which internalize known or putative ligands, and cell transduction facilitating peptides is provided. A ligand displaying genetic package that carries a selectable marker (e.g., reporter, selection, etc.) and presents a ligand on its surface is utilized to identify internalizing ligands, tranduction facilitating peptides, and/or a variety of cells and tissue types for the ability to be successfully transduced by the

ligand displaying genetic package. Also provided are methods for evolving a ligand displaying package to facilitate gene delivery or delivery of any desired agent (e.g., pharmaceutical, polypeptide, peptide, etc.) into a cell and/or targeting cellular compartments such as the nucleus, endosome, chloroplast, mitochondria, etc.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	2002:133421 USPATFULL
TITLE:	Methods using genetic package display for detecting and identifying protein-protein interactions that facilitate internalization and transgene expression and cells or tissues competent for the same and methods for evolving gene delivery vectors
INVENTOR(S):	Larocca, David, Encinitas, CA, UNITED STATES Kassner, Paul, San Mateo, CA, UNITED STATES Baird, Andrew, San Diego, CA, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2002068272	A1	20020606
APPLICATION INFO.:	US 2001-866073	A1	20010524 (9)

RELATED APPLN. INFO.:	Continuation-in-part of Ser. No. WO 2000-US9925361, filed on 25 May 2000, UNKNOWN
DOCUMENT TYPE:	Utility
FILE SEGMENT:	APPLICATION
LEGAL REPRESENTATIVE:	SEED INTELLECTUAL PROPERTY LAW GROUP PLLC, 701 FIFTH AVE, SUITE 6300, SEATTLE, WA, 98104-7092
NUMBER OF CLAIMS:	41
EXEMPLARY CLAIM:	1
NUMBER OF DRAWINGS:	18 Drawing Page(s)
LINE COUNT:	2965

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 21 OF 84 USPATFULL

TI Screening system for **zinc finger** polypeptides for a desired binding ability

AB This invention relates to a **method** for producing a **zinc finger nucleic acid binding protein** comprising preparing a **zinc finger** protein according design rules, varying the protein at one or more positions, and selecting variants which bind to a target nucleic acid sequence by polysome display.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	2002:126312 USPATFULL
TITLE:	Screening system for **zinc finger** polypeptides for a desired binding ability
INVENTOR(S):	Choo, Yen, Cambridge, UNITED KINGDOM Moore, Michael, Amersham Bucks, UNITED KINGDOM

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2002064824	A1	20020530
APPLICATION INFO.:	US 2001-851271	A1	20010508 (9)

RELATED APPLN. INFO.:	Continuation-in-part of Ser. No. WO 1999-GB3730, filed on 9 Nov 1999, UNKNOWN

	NUMBER	DATE
PRIORITY INFORMATION:	GB 1998-24544	19981109

DOCUMENT TYPE:	Utility
FILE SEGMENT:	APPLICATION

LEGAL REPRESENTATIVE: FROMMER LAWRENCE & HAUG LLP, 745 Fifth Avenue, New York, NY, 10151
NUMBER OF CLAIMS: 13
EXEMPLARY CLAIM: 1
LINE COUNT: 1356
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 22 OF 84 USPATFULL
TI 52 human secreted proteins
AB The present invention relates to novel human secreted proteins and isolated nucleic acids containing the coding regions of the genes encoding such proteins. Also provided are vectors, host cells, antibodies, and recombinant methods for producing human secreted proteins. The invention further relates to diagnostic and therapeutic methods useful for diagnosing and treating diseases, disorders, and/or conditions related to these novel human secreted proteins.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2002:126306 USPATFULL
TITLE: 52 human secreted proteins
INVENTOR(S): Ni, Jian, Germantown, MD, UNITED STATES
Baker, Kevin P., Darnestown, MD, UNITED STATES
Birse, Charles E., North Potomac, MD, UNITED STATES
Fiscella, Michele, Bethesda, MD, UNITED STATES
Komatsoulis, George A., Silver Spring, MD, UNITED STATES
Rosen, Craig A., Laytonsville, MD, UNITED STATES
Soppet, Daniel R., Centreville, VA, UNITED STATES
Young, Paul E., Gaithersburg, MD, UNITED STATES
Ebner, Reinhard, Gaithersburg, MD, UNITED STATES
Duan, D. Roxanne, Bethesda, MD, UNITED STATES
Olsen, Henrik S., Gaithersburg, MD, UNITED STATES
LaFleur, David W., Washington, DC, UNITED STATES
Moore, Paul A., Germantown, MD, UNITED STATES
Shi, Yanggu, Gaithersburg, MD, UNITED STATES
Wei, Ping, Brookeville, MD, UNITED STATES
Florence, Kimberly A., Rockville, MD, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2002064818	A1	20020530
APPLICATION INFO.:	US 2001-789561	A1	20010222 (9)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. WO 2000-US24008, filed on 31 Aug 2000, UNKNOWN

	NUMBER	DATE
PRIORITY INFORMATION:	US 1999-152317P	19990903 (60)
	US 1999-152315P	19990903 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: HUMAN GENOME SCIENCES INC, 9410 KEY WEST AVENUE, ROCKVILLE, MD, 20850
NUMBER OF CLAIMS: 23
EXEMPLARY CLAIM: 1
LINE COUNT: 24623
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 23 OF 84 USPATFULL
TI Methods for generating polynucleotides having desired characteristics by iterative selection and recombination
AB A **method** for DNA reassembly after random fragmentation, and

its application to mutagenesis of nucleic acid sequences by in vitro or in vivo recombination is described. In particular, a **method** for the **production** of nucleic acid fragments or polynucleotides encoding mutant proteins is described. The present invention also relates to a **method** of repeated cycles of mutagenesis, shuffling and selection which allow for the directed molecular evolution in vitro or in vivo of proteins.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	2002:122489 USPATFULL
TITLE:	Methods for generating polynucleotides having desired characteristics by iterative selection and recombination
INVENTOR(S):	Stemmer, Willem P. C., Los Gatos, CA, United States
PATENT ASSIGNEE(S):	Maxygen, Inc., Redwood City, CA, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6395547	B1	20020528
APPLICATION INFO.:	US 2000-619550		20000719 (9)

RELATED APPLN. INFO.: Division of Ser. No. US 1999-239395, filed on 28 Jan 1999 Continuation of Ser. No. US 1996-621859, filed on 25 Mar 1996, now patented, Pat. No. US 6117679 Continuation-in-part of Ser. No. US 1995-564955, filed on 30 Nov 1995, now patented, Pat. No. US 5811238 Continuation-in-part of Ser. No. US 537874, now patented, Pat. No. US 5830721 Continuation-in-part of Ser. No. US 1994-198431, filed on 17 Feb 1994, now patented, Pat. No. US 5605793

DOCUMENT TYPE:	Utility
FILE SEGMENT:	GRANTED
PRIMARY EXAMINER:	Whisenant, Ethan C.
LEGAL REPRESENTATIVE:	Kruse, Norman J., Quine, Jonathan Alan, Quine Intellectual Property Law Group, P.C.
NUMBER OF CLAIMS:	42
EXEMPLARY CLAIM:	1
NUMBER OF DRAWINGS:	72 Drawing Figure(s); 37 Drawing Page(s)
LINE COUNT:	6098

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 24 OF 84 USPATFULL

TI Nucleic acids, proteins and antibodies

AB The present invention relates to novel colorectal cancer related polynucleotides, the polypeptides encoded by these polynucleotides herein collectively referred to as "colorectal cancer antigens," and antibodies that immunospecifically bind these polypeptides, and the use of such colorectal cancer polynucleotides, antigens, and antibodies for detecting, treating, preventing and/or prognosing disorders of the colon and/or rectum, including, but not limited to, the presence of colorectal cancer and colorectal cancer metastases. More specifically, isolated colorectal cancer nucleic acid molecules are provided encoding novel colorectal cancer polypeptides. Novel colorectal cancer polypeptides and antibodies that bind to these polypeptides are provided. Also provided are vectors, host cells, and recombinant and synthetic methods for producing human colorectal cancer polynucleotides, polypeptides, and/or antibodies. The invention further relates to diagnostic and therapeutic methods useful for diagnosing, treating, preventing and/or prognosing disorders related to the colon and/or rectum, including colorectal cancer, and therapeutic methods for treating such disorders. The invention further relates to screening methods for identifying agonists and antagonists of polynucleotides and polypeptides of the invention.

The invention further relates to methods and/or compositions for inhibiting or promoting the **production** and/or function of the polypeptides of the invention.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	2002:106416 USPATFULL		
TITLE:	Nucleic acids, proteins and antibodies		
INVENTOR(S):	Rosen, Craig A., Laytonsville, MD, UNITED STATES Ruben, Steven M., Olney, MD, UNITED STATES		
	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2002055627	A1	20020509
APPLICATION INFO.:	US 2001-925299	A1	20010810 (9)
RELATED APPLN. INFO.:	Continuation of Ser. No. WO 2000-US5883, filed on 8 Mar 2000, UNKNOWN		
	NUMBER	DATE	
PRIORITY INFORMATION:	US 1999-124270P	19990312 (60)	
DOCUMENT TYPE:	Utility		
FILE SEGMENT:	APPLICATION		
LEGAL REPRESENTATIVE:	HUMAN GENOME SCIENCES INC, 9410 KEY WEST AVENUE, ROCKVILLE, MD, 20850		
NUMBER OF CLAIMS:	23		
EXEMPLARY CLAIM:	1		
LINE COUNT:	20658		

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 25 OF 84 USPATFULL

TI GTP-binding protein useful in treatment and detection of cancer

AB A novel gene (designated 103P3E8) and its encoded protein are described. While 103P3E8 exhibits tissue specific expression in normal adult tissue, it is aberrantly expressed in multiple cancers including prostate, bladder, kidney, colon, lung, breast, rectal and stomach cancers. Consequently, 103P3E8 provides a diagnostic and/or therapeutic target for cancers, and the 103P3E8 gene or fragment thereof, or its encoded protein or a fragment thereof can be used to elicit an immune response.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	2002:106269 USPATFULL		
TITLE:	GTP-binding protein useful in treatment and detection of cancer		
INVENTOR(S):	Faris, Mary, Los Angeles, CA, UNITED STATES Challita-Eid, Pia M., Encino, CA, UNITED STATES Raitano, Arthur B., Los Angeles, CA, UNITED STATES Mitchell, Steve Chappell, Santa Monica, CA, UNITED STATES Afar, Daniel E.H., Brisbane, CA, UNITED STATES Jakobovits, Aya, Beverly Hills, CA, UNITED STATES		
	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2002055478	A1	20020509
APPLICATION INFO.:	US 2001-834765	A1	20010412 (9)
	NUMBER	DATE	
PRIORITY INFORMATION:	US 2000-196647P	20000412 (60)	
DOCUMENT TYPE:	Utility		
FILE SEGMENT:	APPLICATION		

LEGAL REPRESENTATIVE: GATES & COOPER LLP, HOWARD HUGHES CENTER, 6701 CENTER DRIVE WEST, SUITE 1050, LOS ANGELES, CA, 90045
NUMBER OF CLAIMS: 34
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 18 Drawing Page(s)
LINE COUNT: 5003
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 26 OF 84 USPATFULL
TI Methods for generating polynucleotides having desired characteristics by iterative selection and recombination
AB A **method** for DNA reassembly after random fragmentation, and its application to mutagenesis of nucleic acid sequences by in vitro or in vivo recombination is described. In particular, a **method** for the **production** of nucleic acid fragments or polynucleotides encoding mutant proteins is described. The present invention also relates to a **method** of repeated cycles of mutagenesis, shuffling and selection which allow for the directed molecular evolution in vitro or in vivo of proteins.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2002:81277 USPATFULL
TITLE: Methods for generating polynucleotides having desired characteristics by iterative selection and recombination
INVENTOR(S): Stemmer, Willem P. C., Los Gatos, CA, United States
PATENT ASSIGNEE(S): Maxygen, Inc., Redwood City, CA, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6372497	B1	20020416
APPLICATION INFO.:	US 2000-590774		20000608 (9)

RELATED APPLN. INFO.: Continuation of Ser. No. US 1996-621859, filed on 25 Mar 1996, now patented, Pat. No. US 6117679 Continuation-in-part of Ser. No. US 1995-564955, filed on 30 Nov 1995, now patented, Pat. No. US 5811238 Continuation-in-part of Ser. No. US 537874, now patented, Pat. No. US 5830721 Continuation-in-part of Ser. No. US 1994-198431, filed on 17 Feb 1994, now patented, Pat. No. US 5605793
DOCUMENT TYPE: Utility
FILE SEGMENT: GRANTED
PRIMARY EXAMINER: Whisenant, Ethan
LEGAL REPRESENTATIVE: Kruse, Norman J., Quine, Jonathan Alan, The Law Offices of Jonathan Alan Quine
NUMBER OF CLAIMS: 37
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 72 Drawing Figure(s); 37 Drawing Page(s)
LINE COUNT: 6311
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 27 OF 84 USPATFULL
TI Methods of evolving a polynucleotides by mutagenesis and recombination
AB A **method** of mutating a polynucleotide such that it has a desired or improved functional property is disclosed.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2002:69827 USPATFULL
TITLE: Methods of evolving a polynucleotides by mutagenesis and recombination
INVENTOR(S): Stemmer, Willem P. C., Los Gatos, CA, United States

PATENT ASSIGNEE(S): Maxygen, Inc., Redwood City, CA, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6365408	B1	20020402
APPLICATION INFO.:	US 2000-477763		20000104 (9)

RELATED APPLN. INFO.: Continuation of Ser. No. US 1998-100856, filed on 19 Jun 1998, now patented, Pat. No. US 6132970 Continuation of Ser. No. US 537874, now patented, Pat. No. US 5830721
DOCUMENT TYPE: Utility
FILE SEGMENT: GRANTED
PRIMARY EXAMINER: Whisenant, Ethan
LEGAL REPRESENTATIVE: Kruse, Norman, Liebeschuetz, Joe
NUMBER OF CLAIMS: 40
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 15 Drawing Figure(s); 15 Drawing Page(s)
LINE COUNT: 4167
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 28 OF 84 USPATFULL
TI Exonuclease-mediated nucleic acid reassembly in directed evolution
AB This invention provides methods of obtaining novel polynucleotides and encoded polypeptides by the use of non-stochastic methods of directed evolution (DirectEvolution.TM.). A particular advantage of exonuclease-mediated reassembly methods is the ability to reassemble nucleic acid strands that would otherwise be problematic to chimerize. Exonuclease-mediated reassembly methods can be used in combination with other mutagenesis methods provided herein. These methods include non-stochastic polynucleotide site-saturation mutagenesis (Gene Site Saturation Mutagenesis.TM.) and non-stochastic polynucleotide reassembly (GeneReassembly.TM.). This invention provides methods of obtaining novel enzymes that have optimized physical &/or biological properties. Through use of the claimed methods, genetic vaccines, enzymes, small molecules, and other desirable molecules can be evolved towards desirable properties. For example, vaccine vectors can be obtained that exhibit increased efficacy for use as genetic vaccines. Vectors obtained by using the methods can have, for example, enhanced antigen expression, increased uptake into a cell, increased stability in a cell, ability to tailor an immune response, and the like. Furthermore, this invention provides methods of obtaining a variety of novel biologically active molecules, in the fields of antibiotics, pharmacotherapeutics, and transgenic traits.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2002:63712 USPATFULL
TITLE: Exonuclease-mediated nucleic acid reassembly in directed evolution
INVENTOR(S): Short, Jay M., Rancho Santa Fe, CA, United States
Djavakhishvili, Tsotne David, San Diego, CA, United States
Frey, Gerhard Johann, San Diego, CA, United States
PATENT ASSIGNEE(S): Diversa Corporation, San Diego, CA, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6361974	B1	20020326
APPLICATION INFO.:	US 2000-535754		20000327 (9)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 2000-522289, filed on 9 Mar 2000 Continuation-in-part of Ser. No. US

2000-498557, filed on 4 Feb 2000 Continuation-in-part of Ser. No. US 2000-495052, filed on 31 Jan 2000 Continuation-in-part of Ser. No. US 1999-332835, filed on 14 Jun 1999 Continuation-in-part of Ser. No. US 1999-276860, filed on 26 Mar 1999 Continuation-in-part of Ser. No. US 1999-267118, filed on 9 Mar 1999 Continuation-in-part of Ser. No. US 1999-246178, filed on 4 Feb 1999 Continuation-in-part of Ser. No. US 1998-185373, filed on 3 Nov 1998 Continuation of Ser. No. US 1996-760489, filed on 5 Dec 1996, now patented, Pat. No. US 5830696 Continuation-in-part of Ser. No. US 1997-962504, filed on 31 Oct 1997, now patented, Pat. No. US 6029056 Continuation-in-part of Ser. No. US 1996-677112, filed on 9 Jul 1996, now patented, Pat. No. US 5965408 Continuation-in-part of Ser. No. US 1996-651568, filed on 22 May 1996, now patented, Pat. No. US 5939250

	NUMBER	DATE
PRIORITY INFORMATION:	US 1995-8311P	19951207 (60)
	US 1995-8316P	19951207 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: GRANTED
PRIMARY EXAMINER: Park, Hankyel T.
LEGAL REPRESENTATIVE: Gray Cary Ware & Freidenrich, Haile, Lisa A., Shen, Greg
NUMBER OF CLAIMS: 15
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 6 Drawing Figure(s); 6 Drawing Page(s)
LINE COUNT: 7313
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 29 OF 84 USPATFULL
TI End selection in directed evolution
AB This invention provides methods of obtaining novel polynucleotides and encoded polypeptides by the use of non-stochastic methods of directed evolution (DirectEvolution.TM.). A particular advantage of end-selection-based methods is the ability to recover full-length polynucleotides from a library of progeny molecules generated by mutagenesis methods. These methods include non-stochastic polynucleotide site-saturation mutagenesis (Gene Site Saturation Mutagenesis.TM.) and non-stochastic polynucleotide reassembly (GeneReassembly.TM.). This invention provides methods of obtaining novel enzymes that have optimized physical &/or biological properties. Through use of the claimed methods, genetic vaccines, enzymes, small molecules, and other desirable molecules can be evolved towards desirable properties. For example, vaccine vectors can be obtained that exhibit increased efficacy for use as genetic vaccines. Vectors obtained by using the methods can have, for example, enhanced antigen expression, increased uptake into a cell, increased stability in a cell, ability to tailor an immune response, and the like. Furthermore, this invention provides methods of obtaining a variety of novel biologically active molecules, in the fields of antibiotics, pharmacotherapeutics, and transgenic traits.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2002:57570 USPATFULL
TITLE: End selection in directed evolution
INVENTOR(S): Short, Jay M., Encinitas, CA, United States
Frey, Gerhard Johann, San Diego, CA, United States
PATENT ASSIGNEE(S): Diversa Corporation, San Diego, CA, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6358709	B1	20020319
APPLICATION INFO.:	US 2000-522289		20000309 (9)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 2000-498557, filed on 4 Feb 2000 Continuation-in-part of Ser. No. US 2000-495052, filed on 13 Jan 2000 Continuation-in-part of Ser. No. US 1999-332835, filed on 14 Jun 1999, now abandoned Continuation-in-part of Ser. No. US 1999-276860, filed on 26 Mar 1999 Continuation-in-part of Ser. No. US 1999-267118, filed on 9 Mar 1999, now patented, Pat. No. US 6238884 Continuation-in-part of Ser. No. US 1999-246178, filed on 4 Feb 1999, now patented, Pat. No. US 6171820 Continuation-in-part of Ser. No. US 1998-185373, filed on 3 Nov 1998 Continuation of Ser. No. US 1996-760489, filed on 5 Dec 1996, now patented, Pat. No. US 5830696 Continuation-in-part of Ser. No. US 1997-962504, filed on 31 Oct 1997 Continuation-in-part of Ser. No. US 1996-677112, filed on 9 Jul 1996, now patented, Pat. No. US 5965408 Continuation-in-part of Ser. No. US 1996-651568, filed on 22 May 1996, now patented, Pat. No. US 5939250

	NUMBER	DATE
PRIORITY INFORMATION:	US 1995-8311P	19951207 (60)
	US 1995-8316P	19951207 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: GRANTED
PRIMARY EXAMINER: Park, Hankyel T.
LEGAL REPRESENTATIVE: Gray Cary Ware & Freidenrich LLP, Haile, Lisa A.
NUMBER OF CLAIMS: 36
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 11 Drawing Figure(s); 7 Drawing Page(s)
LINE COUNT: 7029
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 30 OF 84 USPATFULL
TI Human single nucleotide polymorphisms
AB The invention provides nucleic acid segments of the human genome, particularly nucleic acid segments from genes including polymorphic sites. Allele-specific primers and probes hybridizing to regions flanking or containing these sites are also provided. The nucleic acids, primers and probes are used in applications such as phenotype correlations, forensics, paternity testing, medicine and genetic analysis.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2002:55155 USPATFULL
TITLE: Human single nucleotide polymorphisms
INVENTOR(S): Cargill, Michele, Gaithersburg, MD, UNITED STATES
Ireland, James S., Gaithersburg, MD, UNITED STATES
Lander, Eric S., Cambridge, MA, UNITED STATES
PATENT ASSIGNEE(S): Whitehead Institute for Biomedical Research, Cambridge, MA, UNITED STATES (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2002032319	A1	20020314
APPLICATION INFO.:	US 2001-801274	A1	20010307 (9)

	NUMBER	DATE
PRIORITY INFORMATION:	US 2000-187510P	20000307 (60)
	US 2000-206129P	20000522 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: HAMILTON BROOK SMITH AND REYNOLDS, P.C., TWO MILITIA DR, LEXINGTON, MA, 02421-4799
NUMBER OF CLAIMS: 12
EXEMPLARY CLAIM: 1
LINE COUNT: 8981
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 31 OF 84 USPATFULL
TI **METHOD** OF DNA SHUFFLING WITH POLYNUCLEOTIDES PRODUCED BY BLOCKING OR INTERRUPTING A SYNTHESIS OR AMPLIFICATION PROCESS
AB Disclosed is a process of performing "Sexual" PCR which includes generating random polynucleotides by interrupting or blocking a synthesis or amplification process to show or halt synthesis or amplification of at least one polynucleotide, optionally amplifying the polynucleotides, and reannealing the polynucleotides to produce random mutant polynucleotides. Also provided are vector and expression vehicles including such mutant polynucleotides, polypeptides expressed by the mutant polynucleotides and a **method** for producing random mutant polypeptides.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2002:48252 USPATFULL
TITLE: **METHOD** OF DNA SHUFFLING WITH POLYNUCLEOTIDES PRODUCED BY BLOCKING OR INTERRUPTING A SYNTHESIS OR AMPLIFICATION PROCESS
INVENTOR(S): SHORT, JAY M., ENCINITAS, CA, UNITED STATES

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2002028443	A1	20020307	
APPLICATION INFO.:	US 1999-214645	A1	19990927	(9)
	WO 1997-US12239		19970709	

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: LISA A. HAILE PH.D., GRAY CARY WARE & FREIDENRICH LLP, 4365 EXECUTIVE DRIVE, SUITE 1600, SAN DIEGO, CA, 92121
NUMBER OF CLAIMS: 8
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 6 Drawing Page(s)
LINE COUNT: 2551
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 32 OF 84 USPATFULL
TI Exonucease-mediated gene assembly in directed evolution
AB A directed evolution process comprising novel methods for generating improved progeny molecules having desirable properties, including, for example, a **method** for rapid and facilitated **production** from a parental polynucleotide template, of a set of mutagenized progeny polynucleotides wherein at least one codon encoding each of the 20 naturally encoded amino acids is represented at each original codon position. This **method**, termed site-saturation mutagenesis, or simply saturation mutagenesis, is preferably based on the use of the degenerate N,N,G/T sequence. Also, a **method** of producing from a parental polypeptide template, a set of mutagenized progeny polypeptides wherein each of the 20 naturally encoded amino acids is

represented at each original amino acid position. Also, other mutagenization processes that can be used in combination with, or in lieu of, saturation mutagenesis, including, for example: (a) assembly and/or reassembly of polynucloetide building blocks (including sections of genes &/or of gene families) mediated by a source of exonuclease activity such as exonuclease III; and (b) introduction of two or more related polynucleotides into a suitable host cell such that a hybrid polynucleotide is generated by recombination and reductive reassortment. Also molecular property screening methods, including a preferred **method**, termed end selection, comprised of using an enzyme, such as a topoisomerase, a restriction endonuclease, &/or a nicking enzyme (such as N. BstNB I), to detect a specific terminal sequence in a working polynucleotide, to produce a ligatable end thereat, and to ligate and clone the working polynucleotide.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	2002:45482 USPATFULL
TITLE:	Exonucease-mediated gene assembly in directed evolution
INVENTOR(S):	Short, Jay M., Encinitas, CA, United States Frey, Gerhard J., San Diego, CA, United States Djavakhishvili, Tsotne D., San Diego, CA, United States
PATENT ASSIGNEE(S):	Diversa Corporation, San Diego, CA, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6352842	B1	20020305
APPLICATION INFO.:	US 1999-276860		19990326 (9)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 1999-267118, filed on 9 Mar 1999, now patented, Pat. No. US 6238884 Continuation-in-part of Ser. No. US 1999-246178, filed on 4 Feb 1999, now patented, Pat. No. US 6171820 Continuation-in-part of Ser. No. US 1998-185373, filed on 3 Nov 1998 Continuation of Ser. No. US 1996-760489, filed on 5 Dec 1996, now patented, Pat. No. US 5830696 Continuation-in-part of Ser. No. US 1997-962504, filed on 31 Oct 1997, now abandoned Continuation-in-part of Ser. No. US 1996-677112, filed on 9 Jul 1996, now patented, Pat. No. US 5965408 Continuation-in-part of Ser. No. US 1996-651568, filed on 22 May 1996, now patented, Pat. No. US 5939250

	NUMBER	DATE
PRIORITY INFORMATION:	US 1995-8311P	19951207 (60)
	US 1995-8316P	19951207 (60)

DOCUMENT TYPE:	Utility
FILE SEGMENT:	GRANTED
PRIMARY EXAMINER:	Park, Hankyel T.
LEGAL REPRESENTATIVE:	Gray Cary Ware & Freidenrich LLP, Haile, Lisa A., Shen, Greg
NUMBER OF CLAIMS:	20
EXEMPLARY CLAIM:	1
NUMBER OF DRAWINGS:	1 Drawing Figure(s); 1 Drawing Page(s)
LINE COUNT:	4817

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 33 OF 84 USPATFULL

TI Expressed sequences of arabidopsis thaliana

AB Isolated nucleotide compositions and sequences are provided for Arabidopsis thaliana genes. The nucleic acid compositions find use in identifying homologous or related genes; in producing compositions that

modulate the expression or function of its encoded protein, mapping functional regions of the protein; and in studying associated physiological pathways. The genetic sequences may also be used for the genetic manipulation of cells, particularly of plant cells. The encoded gene products and modified organisms are useful for screening of biologically active agents, e.g. fungicides, insecticides, etc.; for elucidating biochemical pathways; and the like.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER: 2002:38558 USPATFULL
TITLE: Expressed sequences of arabidopsis thaliana
INVENTOR(S): Gorlach, Jorn, Durham, NC, UNITED STATES
An, Yong-Qiang, San Diego, CA, UNITED STATES
Hamilton, Carol M., Apex, NC, UNITED STATES
Price, Jennifer L., Raleigh, NC, UNITED STATES
Raines, Tracy M., Durham, NC, UNITED STATES
Yu, Yang, Martinsville, NJ, UNITED STATES
Rameaka, Joshua G., Durham, NC, UNITED STATES
Page, Amy, Durham, NC, UNITED STATES
Mathew, Abraham V., Cary, NC, UNITED STATES
Ledford, Brooke L., Holly Springs, NC, UNITED STATES
Woessner, Jeffrey P., Hillsborough, NC, UNITED STATES
Haas, William David, Durham, NC, UNITED STATES
Garcia, Carlos A., Carrboro, NC, UNITED STATES
Kricker, Maja, Pittsboro, NC, UNITED STATES
Slater, Ted, Apex, NC, UNITED STATES
Davis, Keith R., Durham, NC, UNITED STATES
Allen, Keith, Cary, NC, UNITED STATES
Hoffman, Neil, Chapel Hill, NC, UNITED STATES
Hurban, Patrick, Raleigh, NC, UNITED STATES

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2002023280	A1	20020221	
APPLICATION INFO.:	US 2001-770444	A1	20010126	(9)

	NUMBER	DATE
PRIORITY INFORMATION:	US 2000-178502P	20000127 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: PARADIGM GENETICS, INC, 104 ALEXANDER DRIVE, BUILDING 2, P O BOX 14528, RTP, NC, 277094528
NUMBER OF CLAIMS: 27
EXEMPLARY CLAIM: 1
LINE COUNT: 3845
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 34 OF 84 USPATFULL
TI Methods for recombining nucleic acids
AB A **method** for DNA reassembly after random fragmentation, and its application to mutagenesis of nucleic acid sequences by in vitro or in vivo recombination is described. In particular, a **method** for the **production** of nucleic acid fragments or polynucleotides encoding mutant proteins is described. The present invention also relates to a **method** of repeated cycles of mutagenesis, shuffling and selection which allow for the directed molecular evolution in vitro or in vivo of proteins.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2002:24196 USPATFULL
TITLE: Methods for recombining nucleic acids

INVENTOR(S): Stemmer, Willem P.C., Los Gatos, CA, United States
PATENT ASSIGNEE(S): Maxygen, Inc., Redwood City, CA, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6344356	B1	20020205
APPLICATION INFO.:	US 2000-590778		20000608 (9)

RELATED APPLN. INFO.: Continuation of Ser. No. US 1996-621859, filed on 25 Mar 1996, now patented, Pat. No. US 6117679 Continuation-in-part of Ser. No. US 1995-564955, filed on 30 Nov 1995, now patented, Pat. No. US 5811238 Continuation-in-part of Ser. No. US 537874, now patented, Pat. No. US 5830721 Continuation-in-part of Ser. No. US 1994-198431, filed on 17 Feb 1994, now patented, Pat. No. US 5605793
DOCUMENT TYPE: Utility
FILE SEGMENT: GRANTED
PRIMARY EXAMINER: Whisenant, Ethan
LEGAL REPRESENTATIVE: Kruse, Norman J., Quine, Jonathan Alan, Law Ofices of Jonathan Alan Quine
NUMBER OF CLAIMS: 37
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 72 Drawing Figure(s); 37 Drawing Page(s)
LINE COUNT: 6408
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 35 OF 84 USPATFULL
TI Arrays for identifying agents which mimic or inhibit the activity of interferons
AB Methods and model systems for identifying and characterizing new therapeutic agents, particularly proteins, which mimic or inhibit the activity of all interferons, Type I interferons, IFN-α, IFN-β, or IFN-γ. The **method** comprises administering an interferon selected from the group consisting of IFN-α, IFN β, IFN-τ, IFN-ω, IFN-γ, and combinations thereof to cultured cells, administering the candidate agent to a duplicate culture of cells; and measuring the effect of the candidate agent and the interferon on the transcription or translation of one or, preferably, a plurality of the interferon stimulated genes or the interferon repressed genes (hereinafter referred to as "ISG's" and "IRGs", respectively). The model system is an array with gene probes that hybridize with from about 100 to about 5000 ISG and IRG transcripts.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2001:231143 USPATFULL
TITLE: Arrays for identifying agents which mimic or inhibit the activity of interferons
INVENTOR(S): Silverman, Robert H., Beachwood, OH, United States
Williams, Bryan R. G., Cleveland, OH, United States
Der, Sandy, Cleveland, OH, United States
PATENT ASSIGNEE(S): The Cleveland Clinic Foundation, Cleveland, OH, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6331396	B1	20011218
APPLICATION INFO.:	US 1999-405438		19990923 (9)

	NUMBER	DATE
PRIORITY INFORMATION:	US 1998-101497P	19980923 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: GRANTED
PRIMARY EXAMINER: Zitomer, Stephanie
ASSISTANT EXAMINER: Forman, B J
LEGAL REPRESENTATIVE: Calfee, Halter & Griswold LLP
NUMBER OF CLAIMS: 8
EXEMPLARY CLAIM: 1
LINE COUNT: 9639
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 36 OF 84 USPATFULL
TI Methods for modulating cellular and organismal phenotypes
AB Methods for identifying and controlling the genetic and metabolic pathways underlying complex phenotypes are provided. Conjoint polynucleotide segments that contribute to or disrupt elements of a multigenic phenotype are produced and expressed in cells of interest. Conjoint polynucleotide segments are recombined and/or mutated to give rise to libraries of recombinant concatamers which are expressed in cells of interest. Libraries of conjoint polynucleotide segments and recombinant concatamers are expressed episomally or integrated into the DNA of organelles or chromosomes. Cells are screened or selected to identify members of the population of cells exhibiting a desired phenotype. Libraries and vectors comprising conjoint polynucleotide segments and recombinant concatamers, as well as cells expressing such libraries and vectors or their components are provided. Kits containing conjoint polynucleotide segments, recombinant concatamers, vectors including such polynucleotides, and cells including such polynucleotides and vectors are provided.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2001:223887 USPATFULL
TITLE: Methods for modulating cellular and organismal phenotypes
INVENTOR(S): Stemmer, Willem P.C., Los Gatos, CA, United States
Minshull, Jeremy, Menlo Park, CA, United States
Keenan, Robert J., San Francisco, CA, United States

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2001049104	A1	20011206
APPLICATION INFO.:	US 2001-817015	A1	20010323 (9)

	NUMBER	DATE
PRIORITY INFORMATION:	US 2000-191782P	20000324 (60)
	US 2001-262617P	20010117 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: LAW OFFICES OF JONATHAN ALAN QUINE, P O BOX 458, ALAMEDA, CA, 94501
NUMBER OF CLAIMS: 185
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 6 Drawing Page(s)
LINE COUNT: 3382
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 37 OF 84 USPATFULL
TI Methods for generating polynucleotides having desired characteristics by iterative selection and recombination
AB A **method** for DNA reassembly after random fragmentation, and its application to mutagenesis of nucleic acid sequences by in vitro or in vivo recombination is described. In particular, a **method**

for the **production** of nucleic acid fragments or polynucleotides encoding mutant proteins is described. The present invention also relates to a **method** of repeated cycles of mutagenesis, shuffling and selection which allow for the directed molecular evolution in vitro or in vivo of proteins.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	2001:214886 USPATFULL
TITLE:	Methods for generating polynucleotides having desired characteristics by iterative selection and recombination
INVENTOR(S):	Stemmer, Willem P. C., Los Gatos, CA, United States
PATENT ASSIGNEE(S):	Maxygen, Inc., Redwood City, CA, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6323030	B1	20011127
APPLICATION INFO.:	US 1999-240310		19990129 (9)

RELATED APPLN. INFO.: Continuation of Ser. No. US 1996-621859, filed on 25 Mar 1996, now patented, Pat. No. US 6117679 Continuation-in-part of Ser. No. US 1995-564955, filed on 30 Nov 1995, now patented, Pat. No. US 5811238 Continuation-in-part of Ser. No. US 537874, now patented, Pat. No. US 5830721 Continuation-in-part of Ser. No. US 1994-198431, filed on 17 Feb 1994, now patented, Pat. No. US 5605793

DOCUMENT TYPE:	Utility
FILE SEGMENT:	GRANTED
PRIMARY EXAMINER:	Whisnant, Ethan
LEGAL REPRESENTATIVE:	Kruse, Norman J., Quine, Jonathan AlanThe Law Offices of Jonathan Alan Quine
NUMBER OF CLAIMS:	26
EXEMPLARY CLAIM:	1
NUMBER OF DRAWINGS:	72 Drawing Figure(s); 37 Drawing Page(s)
LINE COUNT:	6066

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 38 OF 84 USPATFULL

TI Chemically assembled nano-scale circuit elements

AB The present invention provides nano-scale devices, including electronic circuits, using DNA molecules as a support structure. DNA binding proteins are used to mask regions of the DNA as a material, such as a metal is coated onto the DNA. Included in the invention are DNA based transistors, capacitors, inductors and diodes. The present invention also provides methods of making integrated circuits using DNA molecules as a support structure. Methods are also included for making DNA based transistors, capacitors, inductors and diodes.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	2001:212120 USPATFULL
TITLE:	Chemically assembled nano-scale circuit elements
INVENTOR(S):	Connolly, Dennis Michael, Rochester, NY, United States
PATENT ASSIGNEE(S):	Integrated Nano-Technologies, LLC. (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2001044114	A1	20011122
APPLICATION INFO.:	US 2001-860046	A1	20010517 (9)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 1999-315750, filed on 20 May 1999, GRANTED, Pat. No. US 6248529

	NUMBER	DATE
PRIORITY INFORMATION:	US 1998-86163P	19980520 (60)
	US 1998-95096P	19980803 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: Gunnar G. Leinberg, NIXON PEABODY LLP, Clinton Square, P.O. Box 31051, Rochester, NY, 14603
NUMBER OF CLAIMS: 71
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 5 Drawing Page(s)
LINE COUNT: 1302
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 39 OF 84 USPATFULL
TI Oligonucleotides which specifically bind retroviral nucleocapsid proteins
AB The invention provides oligonucleotides which bind to retroviral nucleocapsid proteins with high affinity, molecular decoys for retroviral nucleocapsid proteins which inhibit viral replication, targeted molecules comprising high affinity oligonucleotides, assays for selecting test compounds, and related kits.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2001:202380 USPATFULL
TITLE: Oligonucleotides which specifically bind retroviral nucleocapsid proteins
INVENTOR(S): Rein, Alan, Columbia, MD, United States
Casas-Finet, Jose, Gaithersburg, MD, United States
Fisher, Robert, Sharpsburg, MD, United States
Fivash, Matthew, Frederick, MD, United States
Henderson, Louis E., Mount Airy, MD, United States
PATENT ASSIGNEE(S): The United States of America as represented by the Secretary of the Department of Health and Human Services, Washington, DC, United States (U.S. government)

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 6316190	B1	20011113	
	WO 9744064		19971127	
APPLICATION INFO.:	US 1999-180903		19990712	(9)
	WO 1997-US8936		19970519	
			19990712	PCT 371 date
			19990712	PCT 102(e) date

	NUMBER	DATE
PRIORITY INFORMATION:	US 1996-17128P	19960520 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: GRANTED
PRIMARY EXAMINER: Park, Hankyel T.
LEGAL REPRESENTATIVE: Townsend & Townsend & Crew LLP
NUMBER OF CLAIMS: 37
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 8 Drawing Figure(s); 8 Drawing Page(s)
LINE COUNT: 2237
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 40 OF 84 USPATFULL
TI Methods for generating polynucleotides having desired characteristics by iterative selection and recombination

AB A **method** for DNA reassembly after random fragmentation, and its application to mutagenesis of nucleic acid sequences by in vitro or in vivo recombination is described. In particular, a **method** for the **production** of nucleic acid fragments or polynucleotides encoding mutant proteins is described. The present invention also relates to a **method** of repeated cycles of mutagenesis, shuffling and selection which allow for the directed molecular evolution in vitro or in vivo of proteins.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER: 2001:167941 USPATFULL
TITLE: Methods for generating polynucleotides having desired characteristics by iterative selection and recombination
INVENTOR(S): Stemmer, Willem P. C., Los Gatos, CA, United States
PATENT ASSIGNEE(S): Maxygen, Inc., Redwood City, CA, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6297053	B1	20011002
APPLICATION INFO.:	US 2000-501698		20000210 (9)

RELATED APPLN. INFO.: Continuation of Ser. No. US 1998-133508, filed on 12 Aug 1998 Continuation of Ser. No. US 1998-100856, filed on 19 Jun 1998, now patented, Pat. No. US 6132970 Continuation of Ser. No. US 537874, now patented, Pat. No. US 5830721 Continuation-in-part of Ser. No. US 1994-198431, filed on 17 Feb 1994, now patented, Pat. No. US 5605793
DOCUMENT TYPE: Utility
FILE SEGMENT: GRANTED
PRIMARY EXAMINER: Whisenant, Ethan
LEGAL REPRESENTATIVE: Kruse, Esq., Norman J., Quin, Esq., Jonathan AlanLaw Office of Jonathan Alan Quine
NUMBER OF CLAIMS: 26
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 16 Drawing Figure(s); 15 Drawing Page(s)
LINE COUNT: 3937
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 41 OF 84 USPATFULL
TI Methods for generating polynucleotides having desired characteristics by iterative selection and recombination
AB A **method** for DNA reassembly after random fragmentation, and its application to mutagenesis of nucleic acid sequences by in vitro or in vivo recombination is described. In particular, a **method** for the **production** of nucleic acid fragments or polynucleotides encoding mutant proteins is described. The present invention also relates to a **method** of repeated cycles of mutagenesis, shuffling and selection which allow for the directed molecular evolution in vitro or in vivo of proteins.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER: 2001:158074 USPATFULL
TITLE: Methods for generating polynucleotides having desired characteristics by iterative selection and recombination
INVENTOR(S): Stemmer, Willem P. C., Los Gatos, CA, United States
PATENT ASSIGNEE(S): Maxygen, Inc., Redwood City, CA, United States (U.S. corporation)

	NUMBER	KIND	DATE

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6291242	B1	20010918
APPLICATION INFO.:	US 1998-165060		19981002 (9)

RELATED APPLN. INFO.: Continuation of Ser. No. US 1996-621859, filed on 25 Mar 1996, now patented, Pat. No. US 6117679 Continuation-in-part of Ser. No. US 537874, now patented, Pat. No. US 5830721 Continuation-in-part of Ser. No. US 1995-564965, filed on 30 Nov 1995, now patented, Pat. No. US 5811238 Continuation-in-part of Ser. No. US 1994-198431, filed on 17 Feb 1994, now patented, Pat. No. US 5605793

DOCUMENT TYPE: Utility
FILE SEGMENT: GRANTED
PRIMARY EXAMINER: Whisenant, Ethan
LEGAL REPRESENTATIVE: Liebeschuetz, Joe, Kruse, Norman
NUMBER OF CLAIMS: 21
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 72 Drawing Figure(s); 37 Drawing Page(s)
LINE COUNT: 5808
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 42 OF 84 USPATFULL

TI Systematic evolution of ligands by exponential enrichment: photoselection of nucleic acid ligands and solution selex

AB A **method** for identifying nucleic acid ligands to target molecules using the SELEX procedure wherein the candidate nucleic acids contain photoreactive groups and nucleic acid ligands identified thereby are claimed. The complexes of increased affinity nucleic acids and target molecules formed in the procedure are crosslinked by irradiation to facilitate separation from unbound nucleic acids. In other methods partitioning of high and low affinity nucleic acids is facilitated by primer extension steps as shown in the figure in which chain termination nucleotides, digestion resistant nucleotides or nucleotides that allow retention of the cDNA product on an affinity matrix are differentially incorporated into the cDNA products of either the high or low affinity nucleic acids and the cDNA products are treated accordingly to amplification, enzymatic or chemical digestion or by contact with an affinity matrix.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2001:158016 USPATFULL
TITLE: Systematic evolution of ligands by exponential enrichment: photoselection of nucleic acid ligands and solution selex
INVENTOR(S): Gold, Larry, Boulder, CO, United States
Willis, Michael, Louisville, CO, United States
Koch; Tad, Boulder, CO, United States
Ringquist, Steven, Lyons, CO, United States
Jensen, Kirk, Boulder, CO, United States
Atkinson, Brent, Boulder, CO, United States
PATENT ASSIGNEE(S): SomaLogic, Inc., Boulder, CA, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6291184	B1	20010918
APPLICATION INFO.:	US 1999-459553		19991213 (9)

RELATED APPLN. INFO.: Division of Ser. No. US 1998-93293, filed on 8 Jun 1998, now patented, Pat. No. US 6001577 Continuation of Ser. No. US 612895, now patented, Pat. No. US 5763177 Continuation-in-part of Ser. No. US 1993-123935, filed on 17 Sep 1993, now abandoned Continuation-in-part of

Ser. No. US 1993-143564, filed on 25 Oct 1993, now abandoned Continuation-in-part of Ser. No. US 1991-714131, filed on 10 Jun 1991, now patented, Pat. No. US 5475096 Continuation-in-part of Ser. No. US 1990-536428, filed on 11 Jun 1990, now abandoned , said Ser. No. US 612895 Continuation-in-part of Ser. No. US 1992-931473, filed on 17 Aug 1992, now patented, Pat. No. US 5270163 Division of Ser. No. US 714131

DOCUMENT TYPE: Utility
FILE SEGMENT: GRANTED
PRIMARY EXAMINER: Zitomer, Stephanie
LEGAL REPRESENTATIVE: Swanson & Bratschun, L.L.C.
NUMBER OF CLAIMS: 2
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 29 Drawing Figure(s); 35 Drawing Page(s)
LINE COUNT: 2330
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 43 OF 84 USPATFULL
TI Methods for generating polynucleotides having desired characteristics by iterative selection and recombination
AB A **method** for DNA reassembly after random fragmentation, and its application to mutagenesis of nucleic acid sequences by in vitro or in vivo recombination is described. In particular, a **method** for the **production** of nucleic acid fragments or polynucleotides encoding mutant proteins is described. The present invention also relates to a **method** of repeated cycles of mutagenesis, shuffling and selection which allow for the directed molecular evolution in vitro or in vivo of proteins.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2001:152769 USPATFULL
TITLE: Methods for generating polynucleotides having desired characteristics by iterative selection and recombination
INVENTOR(S): Stemmer, Willem P. C., Los Gatos, CA, United States
Crameri, Andreas, Mountain View, CA, United States
PATENT ASSIGNEE(S): Maxygen, Inc., Redwood City, CA, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6287861	B1	20010911
APPLICATION INFO.:	US 1998-133508		19980812 (9)

RELATED APPLN. INFO.: Continuation of Ser. No. US 537874, now patented, Pat. No. US 5830721 Continuation-in-part of Ser. No. US 1994-198431, filed on 17 Feb 1994, now patented, Pat. No. US 5605793
DOCUMENT TYPE: Utility
FILE SEGMENT: GRANTED
PRIMARY EXAMINER: Whisenaut, Ethan
LEGAL REPRESENTATIVE: Liebeschuetz, Joe, Kruse, Norman
NUMBER OF CLAIMS: 94
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 13 Drawing Figure(s); 15 Drawing Page(s)
LINE COUNT: 4081
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 44 OF 84 USPATFULL
TI Methods for generating polynucleotides having desired characteristics by iterative selection and recombination
AB A **method** for DNA reassembly after random fragmentation, and

its application to mutagenesis of nucleic acid sequences by in vitro or in vivo recombination is described. In particular, a **method** for the **production** of nucleic acid fragments or polynucleotides encoding mutant proteins is described. The present invention also relates to a **method** of repeated cycles of mutagenesis, shuffling and selection which allow for the directed molecular evolution in vitro or in vivo of proteins.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	2001:136443 USPATFULL
TITLE:	Methods for generating polynucleotides having desired characteristics by iterative selection and recombination
INVENTOR(S):	Stemmer, Willem P. C., Los Gatos, CA, United States
PATENT ASSIGNEE(S):	Maxygen, Inc., Redwood City, CA, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6277638	B1	20010821
APPLICATION INFO.:	US 1999-232863		19990115 (9)

RELATED APPLN. INFO.:	Division of Ser. No. US 1998-100856, filed on 19 Jun 1998, now patented, Pat. No. US 6132970 Continuation of Ser. No. US 537874, now patented, Pat. No. US 5830721 Continuation-in-part of Ser. No. US 1994-198431, filed on 17 Feb 1994, now patented, Pat. No. US 5605793
DOCUMENT TYPE:	Utility
FILE SEGMENT:	GRANTED
PRIMARY EXAMINER:	Whisenant, Ethan
LEGAL REPRESENTATIVE:	Liebeschuetz, Joe, Kruse, Norman
NUMBER OF CLAIMS:	73
EXEMPLARY CLAIM:	1
NUMBER OF DRAWINGS:	19 Drawing Figure(s); 15 Drawing Page(s)
LINE COUNT:	4027

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 45 OF 84 USPATFULL

TI **Method** of chemically assembling nano-scale devices

AB The present invention provides nano-scale devices, including electronic circuits, using DNA molecules as a support structure. DNA binding proteins are used to mask regions of the DNA as a material, such as a metal is coated onto the DNA. Included in the invention are DNA based transistors, capacitors, inductors and diodes. The present invention also provides methods of making integrated circuits using DNA molecules as a support structure. Methods are also included for making DNA based transistors, capacitors, inductors and diodes.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	2001:93296 USPATFULL
TITLE:	**Method** of chemically assembling nano-scale devices
INVENTOR(S):	Connolly, Dennis Michael, Rochester, NY, United States
PATENT ASSIGNEE(S):	Integrated Nano-Technologies, LLC, Rochester, NY, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6248529	B1	20010619
APPLICATION INFO.:	US 1999-315750		19990520 (9)

NUMBER	DATE

PRIORITY INFORMATION: US 1998-86163P 19980520 (60)
US 1998-95096P 19980803 (60)
DOCUMENT TYPE: Utility
FILE SEGMENT: GRANTED
PRIMARY EXAMINER: Horlick, Kenneth R.
ASSISTANT EXAMINER: Siew, Jeffrey
LEGAL REPRESENTATIVE: Nixon Peabody LLP
NUMBER OF CLAIMS: 32
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 4 Drawing Figure(s); 4 Drawing Page(s)
LINE COUNT: 1011
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 46 OF 84 USPATFULL
TI **Zinc finger** protein derivatives and methods therefor
AB The present invention provides **zinc finger** nucleotide binding polypeptide variants that have at least two **zinc finger** modules that bind to a target cellular nucleotide sequence and modulate the transcriptional function of the cellular nucleotide sequence. Also provided are methods of use of such **zinc finger** nucleotide binding polypeptide variants and methods for isolating the same using expression libraries encoding the polypeptide variants containing randomized substitutions of amino acids. Exemplary **zinc finger** nucleotide binding polypeptide variants of the invention include two cysteines and two histidines whereby both cysteines are amino proximal to both histidines.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2001:82893 USPATFULL
TITLE: **Zinc finger** protein derivatives and methods therefor
INVENTOR(S): Barbas, III, Carlos F., San Diego, CA, United States
Gottesfeld, Joel M., San Diego, CA, United States
Wright, Peter E., La Jolla, CA, United States
PATENT ASSIGNEE(S): The Scripps Research Institute, La Jolla, CA, United States (U.S. corporation)

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 6242568	B1	20010605	
	WO 9519431		19950720	
APPLICATION INFO.:	US 1996-676318		19961230	(8)
	WO 1995-US829		19950118	
			19961230	PCT 371 date
			19961230	PCT 102(e) date

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 1994-312604, filed on 28 Sep 1994, now abandoned Continuation-in-part of Ser. No. US 1994-183119, filed on 18 Jan 1994, now abandoned
DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Achutamurthy, Ponnathapu
ASSISTANT EXAMINER: Moore, William W.
LEGAL REPRESENTATIVE: Gray Cary Ware & Freidenrich LLP, Haile, Lisa A.
NUMBER OF CLAIMS: 56
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 26 Drawing Figure(s); 23 Drawing Page(s)
LINE COUNT: 3179
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 47 OF 84 USPATFULL
TI End selection in directed evolution

AB A directed evolution process comprising novel methods for generating improved progeny molecules having desirable properties, including, for example, a **method** for rapid and facilitated **production** from a parental polynucleotide template, of a set of mutagenized progeny polynucleotides wherein at least one codon encoding each of the 20 naturally encoded amino acids is represented at each original codon position. This **method**, termed site-saturation mutagenesis, or simply saturation mutagenesis, is preferably based on the use of the degenerate N,N,G/T sequence. Also, a **method** of producing from a parental polypeptide template, a set of mutagenized progeny polypeptides wherein each of the 20 naturally encoded amino acids is represented at each original amino acid position. Also, other mutagenization processes that can be used in combination with, or in lieu of, saturation mutagenesis, including, for example: (a) assembly and/or reassembly of polynucloetide building blocks, which building blocks can be sections of genes &/or of gene families; and (b) introduction of two or more related polynucleotides into a suitable host cell such that a hybrid polynucleotide is generated by recombination and reductive reassortment. Also, vector and expression vehicles including such polynucleotides and correspondingly expressed polypeptides. Also molecular property screening methods, including a preferred **method**, termed end selection, comprised of using an enzyme, such as a topoisomerase, a restriction endonuclease, &/or a nicking enzyme (such as N. BstNB I), to detect a specific terminal sequence in a working polynucleotide, to produce a ligatable end thereat, and to ligate and clone the working polynucleotide.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER: 2001:78911 USPATFULL
TITLE: End selection in directed evolution
INVENTOR(S): Short, Jay M., Encinitas, CA, United States
Frey, Gerhard Johann, San Diego, CA, United States
PATENT ASSIGNEE(S): Diversa Corporation, San Diego, CA, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6238884	B1	20010529
APPLICATION INFO.:	US 1999-267118		19990309 (9)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 1999-246178, filed on 4 Feb 1999 Continuation-in-part of Ser. No. US 1998-185373, filed on 3 Nov 1998 Continuation of Ser. No. US 1996-760489, filed on 5 Dec 1996, now patented, Pat. No. US 5830696

	NUMBER	DATE
PRIORITY INFORMATION:	US 1995-8311P	19951207 (60)
	US 1995-8316P	19951207 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Park, Hankyel T.
LEGAL REPRESENTATIVE: Gray Cary Ware & Freidenrich LLP, Haile, Lisa A.
NUMBER OF CLAIMS: 21
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 9 Drawing Figure(s); 5 Drawing Page(s)
LINE COUNT: 4534
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 48 OF 84 USPATFULL
TI Nucleic acid encoding mammalian mu opioid receptor
AB The invention relates generally to compositions of and methods for

obtaining mu opioid receptor polypeptides. The invention relates as well to polynucleotides encoding mu opioid receptor polypeptides, the recombinant vectors carrying those sequences, the recombinant host cells including either the sequences or vectors, and recombinant opioid receptor polypeptides. The invention includes as well, methods for using the isolated, recombinant receptor polypeptide in assays designed to select and improve substances capable of interacting with mu opioid receptor polypeptides for use in diagnostic, drug design and therapeutic applications.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	2001:75149 USPATFULL
TITLE:	Nucleic acid encoding mammalian mu opioid receptor
INVENTOR(S):	Yu, Lei, Indianapolis, IN, United States
PATENT ASSIGNEE(S):	Advanced Research & Technology Institute, Indianapolis, IN, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6235496	B1	20010522
APPLICATION INFO.:	US 1993-120601		19930913 (8)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 1993-56886, filed on 8 Mar 1993, now abandoned

DOCUMENT TYPE:	Utility
FILE SEGMENT:	Granted
PRIMARY EXAMINER:	Kunz, Gary L.
ASSISTANT EXAMINER:	Landsman, Robert S.
LEGAL REPRESENTATIVE:	Fulbright & Jaworski LLP
NUMBER OF CLAIMS:	15
EXEMPLARY CLAIM:	1
NUMBER OF DRAWINGS:	6 Drawing Figure(s); 5 Drawing Page(s)
LINE COUNT:	2811

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 49 OF 84 USPATFULL

TI Methods for generating polynucleotides having desired characteristics by iterative selection and recombination

AB A **method** for DNA reassembly after random fragmentation, and its application to mutagenesis of nucleic acid sequences by in vitro or in vivo recombination is described. In particular, a **method** for the **production** of nucleic acid fragments or polynucleotides encoding mutant proteins is described. The present invention also relates to a **method** of repeated cycles of mutagenesis, shuffling and selection which allow for the directed molecular evolution in vitro or in vivo of proteins.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	2001:14264 USPATFULL
TITLE:	Methods for generating polynucleotides having desired characteristics by iterative selection and recombination
INVENTOR(S):	Stemmer, Willem P.C., Los Gatos, CA, United States
PATENT ASSIGNEE(S):	Maxygen, Inc., Redwood City, CA, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6180406	B1	20010130
APPLICATION INFO.:	US 1998-99015		19980617 (9)

RELATED APPLN. INFO.: Division of Ser. No. US 1996-621859, filed on 25 Mar 1996 Continuation-in-part of Ser. No. US 1995-564955, filed on 30 Nov 1995, now patented, Pat. No. US 5811238

Continuation-in-part of Ser. No. US 537874, now patented, Pat. No. US 5830721 Continuation-in-part of Ser. No. US 1994-198431, filed on 17 Feb 1994, now patented, Pat. No. US 5605793

DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Jones, W. Gary
ASSISTANT EXAMINER: Whisenant, Ethan
LEGAL REPRESENTATIVE: Liebeschuetz, Joe, Kruse, Norman
NUMBER OF CLAIMS: 69
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 37 Drawing Figure(s); 37 Drawing Page(s)
LINE COUNT: 6183
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 50 OF 84 USPATFULL
TI Saturation mutagenesis in directed evolution
AB Disclosed is a rapid and facilitated **method** of producing from a parental template polynucleotide, a set of mutagenized progeny polynucleotides whereby at each original codon position there is produced at least one substitute codon encoding each of the 20 naturally encoded amino acids. Accordingly, there is also provided a **method** of producing from a parental template polypeptide, a set of mutagenized progeny polypeptides wherein each of the 20 naturally encoded amino acids is represented at each original amino acid position. The **method** provided is termed site-saturation mutagenesis, or simply saturation mutagenesis, and can be used in combination with other mutagenization processes, such as, for example, a process wherein two or more related polynucleotides are introduced into a suitable host cell such that a hybrid polynucleotide is generated by recombination and reductive reassortment. Also provided are vector and expression vehicles including such polynucleotides, polypeptides expressed by the hybrid polynucleotides and a **method** for screening for hybrid polypeptides.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2001:4494 USPATFULL
TITLE: Saturation mutagenesis in directed evolution
INVENTOR(S): Short, Jay M., Encinitas, CA, United States
PATENT ASSIGNEE(S): Diversa Corporation, San Diego, CA, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6171820	B1	20010109
APPLICATION INFO.:	US 1999-246178		19990204 (9)

RELATED APPLN. INFO.: Continuation of Ser. No. US 1998-185373, filed on 3 Nov 1998 Continuation-in-part of Ser. No. US 1996-760489, filed on 5 Dec 1996, now patented, Pat. No. US 5830696 Continuation-in-part of Ser. No. US 1997-962504, filed on 31 Oct 1997 Continuation-in-part of Ser. No. US 1996-677112, filed on 9 Jul 1996, now patented, Pat. No. US 5965405, issued on 12 Oct 1999 Continuation-in-part of Ser. No. US 1996-651568, filed on 22 May 1996, now patented, Pat. No. US 5939250, issued on 17 Aug 1999

	NUMBER	DATE
PRIORITY INFORMATION:	US 1995-8311P	19951207 (60)
	US 1995-8316P	19951207 (60)

DOCUMENT TYPE: Patent

FILE SEGMENT: Granted
PRIMARY EXAMINER: Park, Hankyel T.
LEGAL REPRESENTATIVE: Gary Cary Ware & Freidenrich LLP, Haile, Lisa A.
NUMBER OF CLAIMS: 13
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 2 Drawing Figure(s); 2 Drawing Page(s)
LINE COUNT: 3968
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 51 OF 84 USPATFULL
TI Methods for generating polynucleotides having desired characteristics by iterative selection and recombination
AB A **method** for DNA reassembly after random fragmentation, and its application to mutagenesis of nucleic acid sequences by in vitro or in vivo recombination is described. In particular, a **method** for the **production** of nucleic acid fragments or polynucleotides encoding mutant proteins is described. The present invention also relates to a **method** of repeated cycles of mutagenesis, shuffling and selection which allow for the directed molecular evolution in vitro or in vivo of proteins.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2000:174421 USPATFULL
TITLE: Methods for generating polynucleotides having desired characteristics by iterative selection and recombination
INVENTOR(S): Stemmer, Willem P. C., Los Gatos, CA, United States
PATENT ASSIGNEE(S): Maxygen, Inc., Redwood City, CA, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6165793		20001226
APPLICATION INFO.:	US 1998-75511		19980508 (9)

RELATED APPLN. INFO.: Continuation of Ser. No. US 1996-621859, filed on 25 Mar 1996
DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Jones, W. Gary
ASSISTANT EXAMINER: Whisenant, Ethan
LEGAL REPRESENTATIVE: Liebeschuetz, Joe, Kruse, Norman
NUMBER OF CLAIMS: 62
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 37 Drawing Figure(s); 37 Drawing Page(s)
LINE COUNT: 6603
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 52 OF 84 USPATFULL
TI Compositions and methods of use of mammalian retrotransposons
AB The invention relates to an isolated DNAc molecule comprising a promoter P and an L1 cassette sequence comprising a core L1 retrotransposon element and methods of use thereof.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2000:157213 USPATFULL
TITLE: Compositions and methods of use of mammalian retrotransposons
INVENTOR(S): Kazazian, Jr., Haig H., Baltimore, MD, United States
Boeke, Jef D., Baltimore, MD, United States
Moran, John V., Exton, PA, United States
Dombroski, Beth A., Springfield, PA, United States
PATENT ASSIGNEE(S): The John Hopkins University, Baltimore, MD, United

States (U.S. corporation)
The Trustees of the University of Pennsylvania, Philadelphia, PA, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6150160		20001121
APPLICATION INFO.:	US 1997-847844		19970428 (8)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 1996-749805, filed on 15 Nov 1996, now abandoned

	NUMBER	DATE
PRIORITY INFORMATION:	US 1995-6831P	19951116 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Chambers, Jasemine
ASSISTANT EXAMINER: Baker, Anne-Marie
LEGAL REPRESENTATIVE: Akin, Gump, Strauss, Hauer & Feld, L.L.P.
NUMBER OF CLAIMS: 3
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 18 Drawing Figure(s); 33 Drawing Page(s)
LINE COUNT: 3799
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 53 OF 84 USPATFULL
TI **Zinc finger** protein derivatives and methods therefor
AB **Zinc finger** proteins of the Cys.sub.2 His.sub.2 type represent a class of malleable DNA binding proteins which may be selected to bind diverse sequences. Typically, **zinc finger** proteins containing three **zinc finger** domains, like the murine transcription factor Zif268 and the human transcription factor Sp1, bind nine contiguous base pairs (bp). To create a class of proteins which would be generally applicable to target unique sites within complex genomes, the present invention provides a polypeptide linker that fuses two three-finger proteins. Two six-fingered proteins were created and demonstrated to bind 18 contiguous bp of DNA in a sequence specific fashion. Expression of these proteins as fusions to activation or repression domains allows transcription to be specifically up or down modulated within cells. Polydactyl **zinc finger** proteins are broadly applicable as genome-specific transcriptional switches in gene therapy strategies and the development of novel transgenic plants and animals. Such proteins are useful for inhibiting, activating or enhancing gene expression from a **zinc finger**-nucleotide binding motif containing promoter or other transcriptional control element, as well as a structural gene or RNA sequence.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2000:146512 USPATFULL
TITLE: **Zinc finger** protein derivatives and methods therefor
INVENTOR(S): Barbas, III, Carlos F., San Diego, CA, United States
Gottesfeld, Joel M., Del Mar, CA, United States
Wright, Peter E., La Jolla, CA, United States
PATENT ASSIGNEE(S): The Scripps Research Institute, La Jolla, CA, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6140466		20001031
APPLICATION INFO.:	US 1997-863813		19970527 (8)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 676318
DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Achutamurthy, Ponnathapu
ASSISTANT EXAMINER: Moore, William W.
LEGAL REPRESENTATIVE: Gray Cary Ware & Freidenrich LLP, Haile, Lisa A.
NUMBER OF CLAIMS: 54
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 32 Drawing Figure(s); 26 Drawing Page(s)
LINE COUNT: 4196
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 54 OF 84 USPATFULL
TI Methods of shuffling polynucleotides
AB The invention is directed to methods of shuffling polynucleotide variants. The methods entail conducting a multi-cyclic polynucleotide extension process on partially annealed polynucleotide strands having sequences from the plurality of chosen polynucleotide variants, the polynucleotide strands having regions of similarity and regions of heterology with each other and being partially annealed through the regions of similarity, under conditions whereby one strand serves as a template for extension of another strand with which it is partially annealed to generate a population of shuffled polynucleotides. Shuffled polynucleotides are then selected or screened to identify a shuffled polynucleotide having a desired functional property.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2000:138060 USPATFULL
TITLE: Methods of shuffling polynucleotides
INVENTOR(S): Stemmer, Willem P. C., Los Gatos, CA, United States
PATENT ASSIGNEE(S): Maxygen, Inc., Redwood City, CA, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6132970		20001017
APPLICATION INFO.:	US 1998-100856		19980619 (9)

RELATED APPLN. INFO.: Division of Ser. No. US 537874
DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Jones, W. Gary
ASSISTANT EXAMINER: Whisenant, Ethan
LEGAL REPRESENTATIVE: Liebeschuetz, Esq., Joe, Kruse, Esq., Norman
NUMBER OF CLAIMS: 47
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 15 Drawing Figure(s); 15 Drawing Page(s)
LINE COUNT: 4219
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 55 OF 84 USPATFULL
TI Methods for generating polynucleotides having desired characteristics by iterative selection and recombination
AB A **method** for DNA reassembly after random fragmentation, and its application to mutagenesis of nucleic acid sequences by in vitro or in vivo recombination is described. In particular, a **method** for the **production** of nucleic acid fragments or polynucleotides encoding mutant proteins is described. The present invention also relates to a **method** of repeated cycles of mutagenesis, shuffling and selection which allow for the directed molecular evolution in vitro or in vivo of proteins.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER: 2000:121322 USPATFULL
TITLE: Methods for generating polynucleotides having desired characteristics by iterative selection and recombination
INVENTOR(S): Stemmer, Willem P. C., Los Gatos, CA, United States
PATENT ASSIGNEE(S): Maxygen, Inc., Redwood City, CA, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6117679		20000912
APPLICATION INFO.:	US 1996-621859		19960325 (8)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 1995-564955, filed on 30 Nov 1995, now patented, Pat. No. US 5811238 which is a continuation-in-part of Ser. No. US 537874
DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Jones, W. Gary
ASSISTANT EXAMINER: Whisenant, Ethan
LEGAL REPRESENTATIVE: Kruse, Norman J., Liebeschuetz, Joe
NUMBER OF CLAIMS: 35
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 72 Drawing Figure(s); 37 Drawing Page(s)
LINE COUNT: 6708
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 56 OF 84 USPATFULL
TI Polynucleotide encoding mu opioid receptor
AB The invention relates generally to compositions of and methods for obtaining mu opioid receptor polypeptides. The invention relates as well to polynucleotides encoding mu opioid receptor polypeptides, the recombinant vectors carrying those sequences, the recombinant host cells including either the sequences or vectors, recombinant opioid receptor polypeptides, and antibodies immunoreactive with mu opioid receptors. The invention includes as well, methods for using the isolated, recombinant receptor polypeptide in assays designed to select and improve substances capable of interacting with mu opioid receptor polypeptides for use in diagnostic, drug design and therapeutic applications.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2000:105677 USPATFULL
TITLE: Polynucleotide encoding mu opioid receptor
INVENTOR(S): Yu, Lei, Indianapolis, IN, United States
PATENT ASSIGNEE(S): Indiana University, Indianapolis, IN, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6103492		20000815
APPLICATION INFO.:	US 1997-889108		19970707 (8)

RELATED APPLN. INFO.: Continuation of Ser. No. US 1994-305518, filed on 13 Sep 1994, now abandoned which is a continuation-in-part of Ser. No. US 1993-120601, filed on 13 Sep 1993 which is a continuation-in-part of Ser. No. US 1993-56886, filed on 8 Mar 1993, now abandoned
DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Kunz, Gary L.
ASSISTANT EXAMINER: Landsman, Robert
LEGAL REPRESENTATIVE: Fulbright & Jaworski
NUMBER OF CLAIMS: 37

EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 26 Drawing Figure(s); 27 Drawing Page(s)
LINE COUNT: 6028
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 57 OF 84 USPATFULL
TI Compositions containing nucleic acids and ligands for therapeutic treatment
AB Preparations of conjugates of a receptor-binding internalized ligand and a cytocide-encoding agent and compositions containing such preparations are provided. The conjugates contain a polypeptide that is reactive with an FGF receptor, such as bFGF, or another heparin-binding growth factor, cytokine, or growth factor coupled to a nucleic acid binding domain. One or more linkers may be used in the conjugation. The linker is selected to increase the specificity, toxicity, solubility, serum stability, or intracellular availability, and promote nucleic acid condensation of the targeted moiety. The conjugates are complexed with a cytocide-encoding agent, such as DNA encoding saporin. Conjugates of a receptor-binding internalized ligand to a nucleic acid molecule are also provided.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2000:31403 USPATFULL
TITLE: Compositions containing nucleic acids and ligands for therapeutic treatment
INVENTOR(S): Baird, J. Andrew, San Diego, CA, United States
Chandler, Lois Ann, Encinitas, CA, United States
Sosnowski, Barbara A., Coronado, CA, United States
PATENT ASSIGNEE(S): Selective Genetics, Inc., La Jolla, CA, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6037329		20000314
APPLICATION INFO.:	US 1996-718904		19960924 (8)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 1995-441979, filed on 16 May 1995, now abandoned which is a continuation-in-part of Ser. No. US 1994-213446, filed on 15 Mar 1994, now abandoned Ser. No. Ser. No. US 1994-213447, filed on 15 Mar 1994, now abandoned Ser. No. Ser. No. US 1994-297961, filed on 29 Aug 1994, now abandoned And Ser. No. US 1994-305771, filed on 13 Sep 1994, now abandoned
DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Priebe, Scott D.
ASSISTANT EXAMINER: Nguyen, Dave Trong
LEGAL REPRESENTATIVE: Seed and Berry LLP
NUMBER OF CLAIMS: 35
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 34 Drawing Figure(s); 25 Drawing Page(s)
LINE COUNT: 7163
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 58 OF 84 USPATFULL
TI Cobalt Schiff base compounds
AB The invention relates to novel cobalt compounds, having a general structure ##STR1## wherein Co is either Co(II) or Co(III), and each of the R groups is selected from the group consisting of hydrogen, alkyl, hydrophilic organic acid, alkyl amine, amine, alkyl alcohol, alcohol, polypeptide or nucleic acid. The invention further relates to methods of using such compounds to reduce the biological activity of proteins, particularly enzymes and **zinc finger**-containing

proteins.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	1999:170582 USPATFULL
TITLE:	Cobalt Schiff base compounds
INVENTOR(S):	Meade, Thomas J., Altadena, CA, United States Takeuchi, Toshihiko, San Francisco, CA, United States Gray, Harry B., Pasadena, CA, United States Simon, Melvin, San Marino, CA, United States Louie, Angelique Y., Pasadena, CA, United States
PATENT ASSIGNEE(S):	California Institute of Technology, Pasadena, CA, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6008190		19991228
APPLICATION INFO.:	US 1995-570761		19951212 (8)
RELATED APPLN. INFO.:	Continuation-in-part of Ser. No. US 1994-358068, filed on 15 Dec 1994		
DOCUMENT TYPE:	Utility		
FILE SEGMENT:	Granted		
PRIMARY EXAMINER:	Russel, Jeffrey E.		
LEGAL REPRESENTATIVE:	Flehr Hohbach Test Albritton & Herbert LLP, Trecartin, Esq., Richard F., Silva, Esq., Robin M.		
NUMBER OF CLAIMS:	21		
EXEMPLARY CLAIM:	10		
NUMBER OF DRAWINGS:	16 Drawing Figure(s); 6 Drawing Page(s)		
LINE COUNT:	1652		

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 59 OF 84 USPATFULL

TI Systematic evolution of ligands by exponential enrichment: photoselection of nucleic acid ligands and solution selex

AB A **method** for identifying nucleic acid ligands to target molecules using the SELEX procedure wherein the candidate nucleic acids contain photoreactive groups and nucleic acid ligands identified thereby are claimed. The complexes of increased affinity nucleic acids and target molecules formed in the procedure are crosslinked by irradiation to facilitate separation from unbound nucleic acids. In other methods partitioning of high and low affinity nucleic acids is facilitated by primer extension steps as shown in the figure in which chain termination nucleotides, digestion resistant nucleotides or nucleotides that allow retention of the cDNA product on an affinity matrix arc differentially incorporated into the cDNA products of either the high or low affinity nucleic acids and the cDNA products are treated accordingly to amplification, enzymatic or chemical digestion or by contact with an affinity matrix.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	1999:163433 USPATFULL
TITLE:	Systematic evolution of ligands by exponential enrichment: photoselection of nucleic acid ligands and solution selex
INVENTOR(S):	Gold, Larry, Boulder, CO, United States Willis, Michael, Louisville, CO, United States Koch, Tad, Boulder, CO, United States Ringquist, Steven, Lyons, CO, United States Jensen, Kirk, Boulder, CO, United States Atkinson, Brent, Boulder, CO, United States
PATENT ASSIGNEE(S):	NeXstar Pharmaceuticals, Inc., Boulder, CO, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6001577		19991214
APPLICATION INFO.:	US 1998-93293		19980608 (9)

RELATED APPLN. INFO.: Continuation of Ser. No. US 612895
DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Zitomer, Stephanie W.
LEGAL REPRESENTATIVE: Swanson & Bratschun LLC
NUMBER OF CLAIMS: 16
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 29 Drawing Figure(s); 35 Drawing Page(s)
LINE COUNT: 2750
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 60 OF 84 USPATFULL
TI Detection of nucleic acids by multiple sequential invasive cleavages
AB The present invention relates to means for the detection and characterization of nucleic acid sequences, as well as variations in nucleic acid sequences. The present invention also relates to methods for forming a nucleic acid cleavage structure on a target sequence and cleaving the nucleic acid cleavage structure in a site-specific manner. The structure-specific nuclease activity of a variety of enzymes is used to cleave the target-dependent cleavage structure, thereby indicating the presence of specific nucleic acid sequences or specific variations thereof. The present invention further relates to methods and devices for the separation of nucleic acid molecules based on charge. The present invention also provides methods for the detection of non-target cleavage products via the formation of a complete and activated protein binding region. The invention further provides sensitive and specific methods for the detection of human cytomegalovirus nucleic acid in a sample.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 1999:155453 USPATFULL
TITLE: Detection of nucleic acids by multiple sequential invasive cleavages
INVENTOR(S): Hall, Jeff G., Madison, WI, United States
Lyamichev, Victor I., Madison, WI, United States
Mast, Andrea L., Madison, WI, United States
Brow, Mary Ann D., Madison, WI, United States
PATENT ASSIGNEE(S): Third Wave Technologies, Inc., Madison, WI, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 5994069		19991130
APPLICATION INFO.:	US 1997-823516		19970324 (8)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. WO 1997-US1072, filed on 21 Jan 1997 which is a continuation-in-part of Ser. No. US 1996-759038, filed on 2 Dec 1996 And a continuation-in-part of Ser. No. US 1996-758314, filed on 2 Dec 1996 which is a continuation-in-part of Ser. No. US 1996-756386, filed on 26 Nov 1996 which is a continuation-in-part of Ser. No. US 1996-682853, filed on 12 Jul 1996 which is a continuation-in-part of Ser. No. US 1996-599491, filed on 24 Jan 1996 , said Ser. No. US 759038 which is a continuation-in-part of Ser. No. US 1996-756386, filed on 26 Nov 1996
DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Jones, W. Gary

ASSISTANT EXAMINER: Shoemaker, Debra
LEGAL REPRESENTATIVE: Medlen & Carroll, LLP
NUMBER OF CLAIMS: 34
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 169 Drawing Figure(s); 128 Drawing Page(s)
LINE COUNT: 14892
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 61 OF 84 USPATFULL
TI **Method** of DNA reassembly by interrupting synthesis
AB Disclosed is a process of performing Sexual PCR which includes generating random polynucleotides by interrupting or blocking a synthesis or amplification process to show or halt synthesis or amplification of at least one polynucleotide, optionally amplifying the polynucleotides, and reannealing the polynucleotides to produce random mutant polynucleotides. Also provided are vector and expression vehicles including such mutant polynucleotides, polypeptides expressed by the mutant polynucleotides and a **method** for producing random mutant polypeptides.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 1999:124744 USPATFULL
TITLE: **Method** of DNA reassembly by interrupting synthesis
INVENTOR(S): Short, Jay M., Encinitas, CA, United States
PATENT ASSIGNEE(S): Diversa Corporation, San Diego, CA, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 5965408		19991012
APPLICATION INFO.:	US 1996-677112		19960709 (8)

DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Zitomer, Stephanie
LEGAL REPRESENTATIVE: Gray, Cary, Ware & Freidenrich, LLP, Haile, Ph. D., Lisa A.
NUMBER OF CLAIMS: 14
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 2 Drawing Figure(s); 6 Drawing Page(s)
LINE COUNT: 2626
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 62 OF 84 USPATFULL
TI In vitro peptide and antibody display libraries
AB Improved methods and novel compositions for identifying peptides and single-chain antibodies that bind to predetermined receptors or epitopes. Such peptides and antibodies are identified by improved and novel methods for affinity screening of polysomes displaying nascent peptides.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 1999:78552 USPATFULL
TITLE: In vitro peptide and antibody display libraries
INVENTOR(S): Mattheakis, Larry C., Cupertino, CA, United States
Dower, William J., Menlo Park, CA, United States
PATENT ASSIGNEE(S): Affymax Technologies N.V., Greenford, United Kingdom (non-U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 5922545		19990713

APPLICATION INFO.: US 1997-902623 19970729 (8)
RELATED APPLN. INFO.: Continuation of Ser. No. US 1996-586176, filed on 17 Jan 1996, now abandoned which is a continuation-in-part of Ser. No. WO 1994-US12206, filed on 25 Oct 1994, now abandoned which is a continuation-in-part of Ser. No. US 1994-300262, filed on 2 Sep 1994, now abandoned which is a continuation-in-part of Ser. No. US 1993-144775, filed on 29 Oct 1993, now abandoned
DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Wortman, Donna C.
LEGAL REPRESENTATIVE: Stevens, Lauren L., Dunn, Tracy J.
NUMBER OF CLAIMS: 4
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 11 Drawing Figure(s); 8 Drawing Page(s)
LINE COUNT: 3543
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 63 OF 84 USPATFULL
TI Methods for inactivating target DNA and for detecting conformational change in a nucleic acid
AB The present invention reveals a **method** for enzymatically inactivating a target DNA, a **method** for detecting conformational change in a nucleic acid, and a **method** for detecting the presence of a target DNA molecule. The **method** for enzymatically inactivating a target DNA involves preparing a plasmid, phage, virus, or any other delivery vehicle such as a liposome containing a gene encoding a nuclease. The delivery vehicle is then delivered into cells. The cells are induced to produce the nuclease and the target DNA is then enzymatically inactivated. Alternatively, the nuclease protein is delivered directly to cells and used to enzymatically inactivate the target DNA. The **method** for detecting conformational change in a nucleic acid requires contacting a nucleic acid with a hybrid restriction nuclease, determining whether the hybrid restriction nuclease has interacted with the nucleic acid, and detecting the conformational change in the nucleic acid. The **method** for detecting the presence of a target DNA entails contacting a target DNA with a fusion protein, comprising a DNA binding protein joined to a detection domain such as the constant region of an immunoglogulin heavy chain molecule.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 1999:72487 USPATFULL
TITLE: Methods for inactivating target DNA and for detecting conformational change in a nucleic acid
INVENTOR(S): Chandrasegaran, Srinivasan, Baltimore, MD, United States
PATENT ASSIGNEE(S): Johns Hopkins University, Baltimore, MD, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 5916794		19990629
APPLICATION INFO.:	US 1996-647449		19960507 (8)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 1995-575361, filed on 20 Dec 1995, now patented, Pat. No. US 5792640 which is a continuation-in-part of Ser. No. US 1994-346293, filed on 23 Nov 1994, now patented, Pat. No. US 5487994 which is a continuation-in-part of Ser. No. US 1993-126564, filed on 27 Sep 1993, now patented, Pat. No. US 5436150, issued on 25 Jul 1995 which is a continuation-in-part of Ser. No. US 1993-17493, filed

on 12 Feb 1993, now abandoned which is a continuation-in-part of Ser. No. US 1992-862831, filed on 3 Apr 1992, now patented, Pat. No. US 5356802

DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Housel, James C.
ASSISTANT EXAMINER: Swartz, Rodney P.
LEGAL REPRESENTATIVE: Cushman Darby & Cushman, IP Group of Pillsbury, Madison & Sutro
NUMBER OF CLAIMS: 15
EXEMPLARY CLAIM: 1
LINE COUNT: 1533
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 64 OF 84 USPATFULL
TI Programmable genotoxic agents and uses therefor
AB The compositions and methods disclosed herein provide heterobifunctional programmable genotoxic compounds that can be designed to kill selected cells present in a heterogenous cell population. The present compounds comprise a first agent that inflicts damage on cellular DNA, and a second agent that attracts a macromolecular cell component such as a protein, which in turn shields genomic lesions from repair. Unrepaired lesions therefore persist in the cellular genome and contribute to the death of selected cells. In contrast, lesions formed in nonselected cells, which lack the cell component, are unshielded and thus are repaired. As a result, compounds described herein are less toxic to nonselected cells. Compounds of this invention can be designed to cause the selective killing of transformed cells, viral-infected cells and the like.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 1999:33847 USPATFULL
TITLE: Programmable genotoxic agents and uses therefor
INVENTOR(S): Essigmann, John M., Cambridge, MA, United States
Croy, Robert G., Belmont, MA, United States
Chen, Zhenghuan, Malden, MA, United States
PATENT ASSIGNEE(S): Massachusette Institute of Technology, Cambridge, MA, United States (U.S. corporation)

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 5882941		19990316	
APPLICATION INFO.:	US 1994-239428		19940504	(8)

DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Elliott, George C.
ASSISTANT EXAMINER: Brusca, John S.
LEGAL REPRESENTATIVE: Testa Hurwitz & Thibeault, LLP
NUMBER OF CLAIMS: 14
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 14 Drawing Figure(s); 8 Drawing Page(s)
LINE COUNT: 2399
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 65 OF 84 USPATFULL
TI Programmable genotoxic agents and uses therefor
AB The compositions and methods disclosed herein provide heterobifunctional programmable genotoxic compounds that can be designed to kill selected cells present in a heterogenous cell population. The present compounds comprise a first agent that inflicts damage on cellular DNA, and a second agent that attracts a macromolecular cell component such as a protein, which in turn shields genomic lesions from repair. Unrepaired

lesions therefore persist in the cellular genome and contribute to the death of selected cells. In contrast, lesions formed in nonselected cells, which lack the cell component, are unshielded and thus are repaired. As a result, compounds described herein are less toxic to nonselected cells. Compounds of this invention can be designed to cause the selective killing of transformed cells, viral-infected cells and the like.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	1999:30602 USPATFULL		
TITLE:	Programmable genotoxic agents and uses therefor		
INVENTOR(S):	Essigmann, John M., Cambridge, MA, United States Croy, Robert G., Belmont, MA, United States Yarema, Kevin J., Malden, MA, United States Morningstar, Marshall, Cambridge, MA, United States		
PATENT ASSIGNEE(S):	Massachusetts Institute of Technology, Cambridge, MA, United States (U.S. corporation)		
	NUMBER	KIND	DATE
PATENT INFORMATION:	US 5879917		19990309
APPLICATION INFO.:	US 1995-434664		19950504 (8)
RELATED APPLN. INFO.:	Continuation of Ser. No. US 1994-239428, filed on 4 May 1994		
DOCUMENT TYPE:	Utility		
FILE SEGMENT:	Granted		
PRIMARY EXAMINER:	Ketter, James		
ASSISTANT EXAMINER:	Brusca, John S.		
LEGAL REPRESENTATIVE:	Testa Hurwitz & Thibeault,LLP		
NUMBER OF CLAIMS:	19		
EXEMPLARY CLAIM:	1		
NUMBER OF DRAWINGS:	21 Drawing Figure(s); 15 Drawing Page(s)		
LINE COUNT:	2893		

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 66 OF 84 USPATFULL

TI DNA mutagenesis by random fragmentation and reassembly

AB A **method** for generating libraries of displayed peptides and/or antibodies (Abs) suitable for affinity interaction screening or phenotypic screening comprising: (i) obtaining selected library members comprising a displayed peptide and/or Ab and the corressponding polynucleotide (PN), or copies of it, (ii) pooling and fragmenting the PN, or copies of it, to form fragments, (iii) performing PCR amplification and thereby homologously recombining the fragments to form a shuffled pool of recombined PNs, which are not present in the selected library of (i).

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	1998:134871 USPATFULL		
TITLE:	DNA mutagenesis by random fragmentation and reassembly		
INVENTOR(S):	Stemmer, Willem P. C., Los Gatos, CA, United States Crameri, Andreas, Mountain View, CA, United States		
PATENT ASSIGNEE(S):	Affymax Technologies N.V., Curacao, Netherlands Antilles (non-U.S. corporation)		
	NUMBER	KIND	DATE
PATENT INFORMATION:	US 5830721		19981103
	WO 9522625		19950824
APPLICATION INFO.:	US 1996-537874		19960304 (8)
	WO 1995-US2126		19950217
			19960304 PCT 371 date

19960304 PCT 102(e) date

DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Jones, W. Gary
ASSISTANT EXAMINER: Whisenant, Ethan
LEGAL REPRESENTATIVE: Townsend & Townsend & Crew
NUMBER OF CLAIMS: 28
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 15 Drawing Figure(s); 15 Drawing Page(s)
LINE COUNT: 3865
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 67 OF 84 USPATFULL
TI Control of gene expression by ionizing radiation
AB This invention relates to genetic constructs which comprise an enhancer-promoter region which is responsive to radiation, and at least one structural gene whose expression is controlled by the enhancer-promoter. This invention also relates to methods of destroying, altering, or inactivating cells in target tissue by delivering the genetic constructs to the cells of the tissues and inducing expression of the structural gene or genes in the construct by exposing the tissues to ionizing radiation. This invention is useful for treating patients with cancer, clotting disorders, myocardial infarction, and other diseases for which target tissues can be identified and for which gene expression of the construct within the target tissues can alleviate the disease or disorder.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 1998:122387 USPATFULL
TITLE: Control of gene expression by ionizing radiation
INVENTOR(S): Weichselbaum, Ralph R., Chicago, IL, United States
Hallahan, Dennis E., Chicago, IL, United States
Sukhatme, Vikas P., Chicago, IL, United States
Kufe, Donald W., Wellesley, MA, United States
PATENT ASSIGNEE(S): Arch Development Corp., Chicago, IL, United States (U.S. corporation)
Dana-Farber Cancer Institute, Boston, MA, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 5817636		19981006
APPLICATION INFO.:	US 1995-486338		19950607 (8)

RELATED APPLN. INFO.: Continuation of Ser. No. US 1994-212308, filed on 14 Mar 1994, now patented, Pat. No. US 5612318 which is a continuation of Ser. No. US 1993-35897, filed on 18 Mar 1993, now abandoned which is a continuation of Ser. No. US 1990-633626, filed on 20 Dec 1990, now abandoned
DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Campell, Bruce R.
LEGAL REPRESENTATIVE: Arnold, White & Durkee
NUMBER OF CLAIMS: 32
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 21 Drawing Figure(s); 10 Drawing Page(s)
LINE COUNT: 1391
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 68 OF 84 USPATFULL
TI Methods for generating polynucleotides having desired characteristics by iterative selection and recombination
AB A **method** for DNA reassembly after random fragmentation, and

its application to mutagenesis of nucleic acid sequences by in vitro or in vivo recombination is described. In particular, a **method** for the **production** of nucleic acid fragments or polynucleotides encoding mutant proteins is described. The present invention also relates to a **method** of repeated cycles of mutagenesis, shuffling and selection which allow for the directed molecular evolution in vitro or in vivo of proteins.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	1998:115555 USPATFULL
TITLE:	Methods for generating polynucleotides having desired characteristics by iterative selection and recombination
INVENTOR(S):	Stemmer, Willem P. C., Los Gatos, CA, United States Crameri, Andreas, Mountain View, CA, United States
PATENT ASSIGNEE(S):	Affymax Technologies N.V., De Ruyderkade, Netherlands Antilles (non-U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 5811238		19980922
APPLICATION INFO.:	US 1995-564955		19951130 (8)
RELATED APPLN. INFO.:	Continuation-in-part of Ser. No. US 1994-198431, filed on 17 Feb 1994 And Ser. No. US 1996-537874, filed on 4 Mar 1996		
DOCUMENT TYPE:	Utility		
FILE SEGMENT:	Granted		
PRIMARY EXAMINER:	Jones, W. Gary		
ASSISTANT EXAMINER:	Whisenant, Ethan		
LEGAL REPRESENTATIVE:	Townsend & Townsend & Crew		
NUMBER OF CLAIMS:	22		
EXEMPLARY CLAIM:	1		
NUMBER OF DRAWINGS:	22 Drawing Figure(s); 22 Drawing Page(s)		
LINE COUNT:	4466		

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 69 OF 84 USPATFULL

TI Systematic evolution of ligands by exponential enrichment: photoselection of nucleic acid ligands and solution selex

AB A **method** for identifying nucleic acid ligands to target molecules using the SELEX pocedure wherein the candidate nucleic acids contain photoreactive groups and nucleic acid ligands identified thereby are claimed. The complexes of increased affinity nucleic acids and target molecules formed in the procedure are crosslinked by irradiation to facilitate separation from unbound nucleic acids. In other methods partioning of high and low affinity nucleic acids is facilitated by primer extension steps as shown in the figure in which chain termination nucleotides, digestion resistant nucleotides or nucleotides that allow retention of the cDNA product on an affinity matrix are differentially incorporated into the cDNA products of either the high or low affinity nucleic acids and the cDNA products are treated accordingly to amplification, enzymatic or chemical digestion or by contact with an affinity matrix.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	1998:64969 USPATFULL
TITLE:	Systematic evolution of ligands by exponential enrichment: photoselection of nucleic acid ligands and solution selex
INVENTOR(S):	Gold, Larry, Boulder, CO, United States Willis, Michael, Louisville, CO, United States Koch, Tad, Boulder, CO, United States

	Ringquist, Steven, Lyons, CO, United States Jensen, Kirk, Boulder, CO, United States Atkinson, Brent, Boulder, CO, United States
PATENT ASSIGNEE(S):	NeXstar Pharmaceuticals, Inc., Boulder, CO, United States (U.S. corporation)

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 5763177		19980609	
	WO 9508003		19950323	
APPLICATION INFO.:	US 1996-612895		19960308	(8)
	WO 1994-US10562		19940916	
			19960308	PCT 371 date
			19960308	PCT 102(e) date

RELATED APPLN. INFO.:	Continuation-in-part of Ser. No. US 1993-143564, filed on 25 Oct 1993, now abandoned And Ser. No. US 1993-123935, filed on 17 Sep 1993, now abandoned which is a continuation-in-part of Ser. No. US 1991-714131, filed on 10 Jun 1991, now patented, Pat. No. US 5475096 which is a continuation-in-part of Ser. No. US 1990-536428, filed on 11 Jun 1990, now abandoned , said Ser. No. US -143564 which is a continuation-in-part of Ser. No. US -714131 And Ser. No. US 1992-931473, filed on 17 Aug 1992, now patented, Pat. No. US 5270163, issued on 14 Dec 1993
DOCUMENT TYPE:	Utility
FILE SEGMENT:	Granted
PRIMARY EXAMINER:	Zitomer, Stephanie W.
LEGAL REPRESENTATIVE:	Swanson & Bratschun LLC
NUMBER OF CLAIMS:	16
EXEMPLARY CLAIM:	1
NUMBER OF DRAWINGS:	29 Drawing Figure(s); 35 Drawing Page(s)
LINE COUNT:	2714

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 70 OF 84 USPATFULL

TI **Method** and device for diagnosing and distinguishing chest pain in early onset thereof

AB A diagnostic test, and a device for conducting the test, for assessing whether patient chest pain is cardiac in origin and for differentiating between unstable angina and myocardial infarction as a cause of patient chest pain is described. The diagnostic test comprises simultaneously detecting at least three selected cardiac markers with the use of at least three different monoclonal or polyclonal antibody pairs, each member of which is complementary to a different marker, which is released by heart muscle at varying stages after the onset of chest pain and is indicative of the cause of the chest pain.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	1998:48195 USPATFULL
TITLE:	**Method** and device for diagnosing and distinguishing chest pain in early onset thereof
INVENTOR(S):	Jackowski, George, Inglewood, Canada
PATENT ASSIGNEE(S):	Spectral Diagnostics Inc., Toronto, Canada (non-U.S. corporation)

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 5747274		19980505	
APPLICATION INFO.:	US 1996-697690		19960905	(8)

RELATED APPLN. INFO.:	Continuation of Ser. No. US 1995-420298, filed on 11 Apr 1995, now patented, Pat. No. US 5604105 which is a

continuation-in-part of Ser. No. US 1993-26453, filed on 3 Mar 1993, now abandoned which is a continuation-in-part of Ser. No. US 1991-695381, filed on 3 May 1991, now patented, Pat. No. US 5290678, issued on 1 Mar 1994

	NUMBER	DATE
PRIORITY INFORMATION:	CA 1990-2027434	19901012

DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Wolski, Susan
LEGAL REPRESENTATIVE: Klauber & Jackson
NUMBER OF CLAIMS: 25
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 16 Drawing Figure(s); 10 Drawing Page(s)
LINE COUNT: 2438
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 71 OF 84 USPATFULL
TI **Method** and device for diagnosing and distinguishing chest pain in early onset thereof
AB A diagnostic test, and a device for conducting the test, for assessing whether patient chest pain is cardiac in origin and for differentiating between unstable angina and myocardial infarction as a cause of patient chest pain is described. The diagnostic test comprises simultaneously detecting at least three selected cardiac markers with the use of at least three different monoclonal or polyclonal antibody pairs, each member of which is complementary to a different marker, which is released by heart muscle at varying stages after the onset of chest pain and is indicative of the cause of the chest pain.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 1998:45097 USPATFULL
TITLE: **Method** and device for diagnosing and distinguishing chest pain in early onset thereof
INVENTOR(S): Jackowski, George, Inglewood, Canada
PATENT ASSIGNEE(S): Spectral Diagnostics Inc., Toronto, Canada (non-U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 5744358		19980428
APPLICATION INFO.:	US 1996-707594		19960905 (8)

RELATED APPLN. INFO.: Continuation of Ser. No. US 1995-420298, filed on 11 Apr 1995, now patented, Pat. No. US 5604105 which is a continuation-in-part of Ser. No. US 1993-26453, filed on 3 Mar 1993, now abandoned which is a continuation-in-part of Ser. No. US 1991-695381, filed on 3 May 1991, now patented, Pat. No. US 5290678, issued on 1 Mar 1994

	NUMBER	DATE
PRIORITY INFORMATION:	CA 1990-2027434	19901012

DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Wolski, Susan
LEGAL REPRESENTATIVE: Klauber & Jackson
NUMBER OF CLAIMS: 13
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 16 Drawing Figure(s); 10 Drawing Page(s)

LINE COUNT: 2396
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 72 OF 84 USPATFULL
TI High affinity HIV-1 gag nucleic acid ligands
AB Methods are described for the identification and preparation of high-affinity nucleic acid ligands to HIV-1 GAG, Included in the invention are specific RNA ligands to HIV-1 GAG identified by the SELEX **method**, Also included are RNA ligands that inhibit the function of HIV-1 GAG.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 1998:25078 USPATFULL
TITLE: High affinity HIV-1 gag nucleic acid ligands
INVENTOR(S): Lochrie, Michael A., Boulder, CO, United States
Gold, Larry, Boulder, CO, United States
PATENT ASSIGNEE(S): NeXstar Pharmaceuticals, Inc., Boulder, CO, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 5726017		19980310
APPLICATION INFO.:	US 1995-447172		19950519 (8)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 1991-714131, filed on 10 Jun 1991, now patented, Pat. No. US 5475096 , said Ser. No. US 1992-931473, filed on 17 Aug 1992, now patented, Pat. No. US 5270163 , said Ser. No. US 1992-964624, filed on 21 Oct 1992, now patented, Pat. No. US 5496938 , said Ser. No. US 1993-117991, filed on 8 Sep 1993, now abandoned And Ser. No. US 1990-536428, filed on 11 Jun 1990, now abandoned
DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Zitomer, Stephanie W.
LEGAL REPRESENTATIVE: Swanson & Bratschun LLC
NUMBER OF CLAIMS: 10
EXEMPLARY CLAIM: 1
LINE COUNT: 1124
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 73 OF 84 USPATFULL
TI **Method** and device for diagnosing and distinguishing chest pain in early onset thereof
AB This invention relates to a diagnostic tests and devices for conducting such tests at the point of care or in a diagnostic laboratory for accurate, simple, and rapid assessment of chest pain. In particular, the invention relates to differential diagnosis of the origin of chest pain, e.g., whether the pain is cardiac in origin, and for differentiating between unstable angina ("UA"), myocardial infarction ("MI"), congestive heart failure ("CHF"), and other ischemic events affecting the heart, at early onset of patient chest pain. The invention further relates to diagnosis of the stage of the MI in a patient suffering from MI, and to prognosis of such a patient. The present invention allows for the rapid, accurate, and sensitive diagnosis of a cardiac ischemic event in a patient complaining of chest pain, and determination of whether the event is unstable angina or myocardial infarction, by detecting the presence or absence of increased levels of at least three, and preferably four, biochemical markers present in blood or a blood fraction (serum, plasma) from a patient. The biochemical markers are heart proteins released during the ischemia. Release of different proteins occurs at different times and with different levels of ischemia.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	1998:6930 USPATFULL
TITLE:	**Method** and device for diagnosing and distinguishing chest pain in early onset thereof
INVENTOR(S):	Jackowski, George, Inglewood, Canada
PATENT ASSIGNEE(S):	Spectral Diagnostics Inc., Toronto, Canada (non-U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 5710008		19980120
APPLICATION INFO.:	US 1996-735178		19961022 (8)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 1995-420298, filed on 11 Apr 1995, now patented, Pat. No. US 5604105 which is a continuation-in-part of Ser. No. US 1993-26453, filed on 3 Mar 1993, now abandoned which is a continuation-in-part of Ser. No. US 1991-695381, filed on 3 May 1991, now patented, Pat. No. US 5290678, issued on 1 Mar 1994

	NUMBER	DATE
PRIORITY INFORMATION:	CA 1990-2027434	19901012

DOCUMENT TYPE:	Utility
FILE SEGMENT:	Granted
PRIMARY EXAMINER:	Wolski, Susan
LEGAL REPRESENTATIVE:	Klauber & Jackson
NUMBER OF CLAIMS:	34
EXEMPLARY CLAIM:	23
NUMBER OF DRAWINGS:	16 Drawing Figure(s); 10 Drawing Page(s)
LINE COUNT:	2559

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 74 OF 84 USPATFULL

TI High affinity HIV Nucleocapsid nucleic acid ligands

AB Methods are described for the identification and preparation of high-affinity nucleic acid ligands to HIV-1 nucleocapsid. Included in the invention are specific RNA ligands to HIV-1 nucleocapsid identified by the SELEX **method**. Also included are RNA ligands that inhibit the function of HIV-1 nucleocapsid.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	97:68325 USPATFULL
TITLE:	High affinity HIV Nucleocapsid nucleic acid ligands
INVENTOR(S):	Allen, Patrick Nikita, Boulder, CO, United States Gold, Larry, Boulder, CO, United States
PATENT ASSIGNEE(S):	NeXstar Pharmaceuticals, Inc., Boulder, CO, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 5654151		19970805
APPLICATION INFO.:	US 1995-477830		19950607 (8)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 1991-714131, filed on 10 Jun 1991, now patented, Pat. No. US 5475096 Ser. No. Ser. No. US 1992-931473, filed on 17 Aug 1992, now patented, Pat. No. US 5270163 Ser. No. Ser. No. US 1992-964624, filed on 21 Oct 1992, now patented, Pat. No. US 5496938 Ser. No. Ser. No. US 1993-117991, filed on 8 Sep 1993, now abandoned Ser. No. Ser. No. US 1994-361795, filed on 21 Dec 1994 And Ser. No. US

1995-447172, filed on 19 May 1995 , said Ser. No. US -714131 which is a continuation-in-part of Ser. No. US 1990-536428, filed on 11 Jun 1990, now abandoned

DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Zitomer, Stephanie W.
LEGAL REPRESENTATIVE: Swanson & Bratschun LLC
NUMBER OF CLAIMS: 8
EXEMPLARY CLAIM: 1
LINE COUNT: 1190
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 75 OF 84 USPATFULL
TI High affinity HIV nucleocapsid nucleic acid ligands
AB Methods are described for the identification and preparation of high-affinity nucleic acid ligands to HIV-1 nucleocapsid. Included in the invention are specific RNA ligands to HIV-1 nucleocapsid identified by the SELEX **method** and RNA ligands that inhibit the function of HIV-1 nucleocapsid.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 97:47519 USPATFULL
TITLE: High affinity HIV nucleocapsid nucleic acid ligands
INVENTOR(S): Allen, Patrick, Boulder, CO, United States
Gold, Larry, Boulder, CO, United States
PATENT ASSIGNEE(S): NeXstar Pharmaceuticals, Inc., Boulder, CO, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 5635615		19970603
APPLICATION INFO.:	US 1995-477530		19950607 (8)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 1991-714131, filed on 10 Jun 1991, now patented, Pat. No. US 5475096 Ser. No. Ser. No. US 1992-931473, filed on 17 Aug 1992, now patented, Pat. No. US 5270163 Ser. No. Ser. No. US 1992-964624, filed on 21 Oct 1992, now patented, Pat. No. US 5496938 Ser. No. Ser. No. US 1993-117991, filed on 8 Sep 1993, now abandoned Ser. No. Ser. No. US 1994-361795, filed on 21 Dec 1994 And Ser. No. US 1995-447172, filed on 19 May 1995 , said Ser. No. US -714131 which is a continuation-in-part of Ser. No. US 1990-536428, filed on 11 Jun 1990, now abandoned

DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Zitomer, Stephanie W.
LEGAL REPRESENTATIVE: Swanson & Bratschun, LLC
NUMBER OF CLAIMS: 7
EXEMPLARY CLAIM: 1
LINE COUNT: 1191
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 76 OF 84 USPATFULL
TI Control of gene expression by ionizing radiation
AB This invention relates to genetic constructs which comprise an enhancer-promoter region which is responsive to radiation, and at least one structural gene whose expression is controlled by the enhancer-promoter. This invention also relates to methods of destroying, altering, or inactivating cells in target tissue by delivering the genetic constructs to the cells of the tissues and inducing expression of the structural gene or genes in the construct by exposing the tissues to ionizing radiation. This invention is useful for treating patients

with cancer, clotting disorders, myocardial infarction, and other diseases for which target tissues can be identified and for which gene expression of the construct within the target tissues can alleviate the disease or disorder.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	97:22761 USPATFULL
TITLE:	Control of gene expression by ionizing radiation
INVENTOR(S):	Weichselbaum, Ralph R., 2031 N. Sedgwick, Chicago, IL, United States 60616 Hallahan, Dennis E., 5343 N. Moody, Chicago, IL, United States 60630 Sukhatme, Vikas P., 1511 E. 56th St., Chicago, IL, United States 60637 Kufe, Donald W., 179 Grove St., Wellesley, MA, United States 02181

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 5612318		19970318
APPLICATION INFO.:	US 1994-212308		19940314 (8)

RELATED APPLN. INFO.:	Continuation of Ser. No. US 1993-35897, filed on 16 Mar 1993, now abandoned which is a continuation of Ser. No. US 1990-633626, filed on 20 Dec 1990, now abandoned
DOCUMENT TYPE:	Utility
FILE SEGMENT:	Granted
PRIMARY EXAMINER:	Campell, Bruce R.
LEGAL REPRESENTATIVE:	Arnold, White & Durkee
NUMBER OF CLAIMS:	8
EXEMPLARY CLAIM:	1
NUMBER OF DRAWINGS:	21 Drawing Figure(s); 16 Drawing Page(s)
LINE COUNT:	1211

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 77 OF 84 USPATFULL

TI **Method** and device for diagnosing and distinguishing chest pain in early onset thereof

AB A diagnostic test, and a device for conducting the test, for assessing whether patient chest pain is cardiac in origin and for differentiating between unstable angina and myocardial infarction as a cause of patient chest pain is described. The diagnostic test comprises simultaneously detecting at least three selected cardiac markers with the use of at least three different monoclonal or polyclonal antibody pairs, each member of which is complementary to a different marker, which is released by heart muscle at varying stages after the onset of chest pain and is indicative of the cause of the chest pain.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	97:14582 USPATFULL
TITLE:	**Method** and device for diagnosing and distinguishing chest pain in early onset thereof
INVENTOR(S):	Jackowski, George, Inglewood, Canada
PATENT ASSIGNEE(S):	Spectral Diagnostics Inc., Toronto, Canada (non-U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 5604105		19970218
APPLICATION INFO.:	US 1995-420298		19950411 (8)

DISCLAIMER DATE:	20110503
RELATED APPLN. INFO.:	Continuation-in-part of Ser. No. US 1993-26453, filed on 3 Mar 1993, now abandoned which is a

continuation-in-part of Ser. No. US 1991-695381, filed on 3 May 1991, now patented, Pat. No. US 5290678, issued on 1 Mar 1994

	NUMBER	DATE
PRIORITY INFORMATION:	CA 1990-2027434	19901012

DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Knode, Marian C.
ASSISTANT EXAMINER: Wolski, Susan C.
LEGAL REPRESENTATIVE: Klauber & Jackson
NUMBER OF CLAIMS: 23
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 16 Drawing Figure(s); 10 Drawing Page(s)
LINE COUNT: 2462
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 78 OF 84 USPATFULL

TI Chimeric immunogenic gag-V3 virus-like particles of the human immunodeficiency virus (HIV)

AB An unprocessed human immunodeficiency virus 2 (HIV-2) gag precursor protein, containing a deficient protease, assembles into virus-like particles by budding through the cytoplasmic domain of baculovirus-infected cells. Chimeric constructs were generated by coupling the truncated HIV-2 gag gene to the neutralizing domain (V3) or the neutralizing and CD4 binding domains (V3+CD4B) of gp120 env gene sequences obtained from HIV-1 or HIV-2. Virus-like particles were formed by chimeric gene products when the env gene sequences were linked to the 3' terminus of the gag gene. The gag-env chimeric proteins displayed immunoreactivity towards anti-gp120 rabbit antisera.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 96:111363 USPATFULL
TITLE: Chimeric immunogenic gag-V3 virus-like particles of the human immunodeficiency virus (HIV)
INVENTOR(S): Kang, Chil-Yong, London, Canada
Luo, Lizhong, London, Canada
PATENT ASSIGNEE(S): Korea Green Cross Corporation, Kyongki-Do, Korea, Republic of (non-U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 5580773		19961203
APPLICATION INFO.:	US 1993-100118		19930730 (8)
RELATED APPLN. INFO.:	Continuation-in-part of Ser. No. US 1992-992618, filed on 18 Dec 1992		

	NUMBER	DATE
PRIORITY INFORMATION:	KR 1992-10493	19920617

DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Budens, Robert D.
ASSISTANT EXAMINER: Parkin, Jeffrey S.
LEGAL REPRESENTATIVE: Merchant, Gould, Smith, Edell, Welter, & Schmidt
NUMBER OF CLAIMS: 8
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 32 Drawing Figure(s); 18 Drawing Page(s)
LINE COUNT: 848
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 79 OF 84 USPATFULL
TI Chimeric HIV-2 gag particles
AB The chimeric proteins, and a protential vaccine and diagnostic reagent comprising gag-env chimeric protein particles are disclosed. The preparation comprises linking gag of HIV-2 to env to form the chimeric gene, inserting the obtained chimeric gene into the DNA of a baculovirus, infecting insect cells or insect host with the resulting recombinant virus, culturing it and purifying the obtained chimeric protein. The gag chimeric protein of HIV according to the present invention retains both antigenic and immunogenic properties.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	95:75739 USPATFULL		
TITLE:	Chimeric HIV-2 gag particles		
INVENTOR(S):	Kang, Chil-Yong, London, Canada Luo, Lizhong, London, Canada		
PATENT ASSIGNEE(S):	Korea Green Cross Corporation, Korea, Republic of (non-U.S. corporation) a part interest		
	NUMBER	KIND	DATE
PATENT INFORMATION:	US 5443828		19950822
APPLICATION INFO.:	US 1992-992618		19921218 (7)
	NUMBER		DATE
PRIORITY INFORMATION:	KR 1992-10493		19920617
DOCUMENT TYPE:	Utility		
FILE SEGMENT:	Granted		
PRIMARY EXAMINER:	Nucker, Christine M.		
ASSISTANT EXAMINER:	Tuscan, M.		
LEGAL REPRESENTATIVE:	Merchant, Gould, Smith, Edell, Welter & Schmidt		
NUMBER OF CLAIMS:	4		
EXEMPLARY CLAIM:	1		
NUMBER OF DRAWINGS:	19 Drawing Figure(s); 11 Drawing Page(s)		
LINE COUNT:	621		

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 80 OF 84 USPATFULL
TI Molecular clones of bovine immunodeficiency-like virus
AB Biologically active proviral molecular clones of bovine immunodeficiency-like virus and cell lines infected with the same have been prepared. Various utilities of the clones are described.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	95:3945 USPATFULL		
TITLE:	Molecular clones of bovine immunodeficiency-like virus		
INVENTOR(S):	Gonda, Matthew A., Walkersville, MD, United States		
PATENT ASSIGNEE(S):	The United States of America as represented by the Secretary of the Department of Health and Human Services, Washington, DC, United States (U.S. government)		
	NUMBER	KIND	DATE
PATENT INFORMATION:	US 5380830		19950110
APPLICATION INFO.:	US 1992-980324		19921124 (7)
RELATED APPLN. INFO.:	Continuation of Ser. No. US 1989-408815, filed on 18 Sep 1989, now abandoned		
DOCUMENT TYPE:	Utility		
FILE SEGMENT:	Granted		
PRIMARY EXAMINER:	Stone, Jacqueline		

ASSISTANT EXAMINER: Railey, II, Johnny F.
LEGAL REPRESENTATIVE: Rucker, Susan S.
NUMBER OF CLAIMS: 3
EXEMPLARY CLAIM: 2
NUMBER OF DRAWINGS: 40 Drawing Figure(s); 28 Drawing Page(s)
LINE COUNT: 1180
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 81 OF 84 DGENE (C) 2002 THOMSON DERWENT
TI Use of a nucleic acid binding polypeptide capable of binding to telomeric, G-quadruplex, or G-quartet nucleic acid as an enzymatic activity inhibitor or cytotoxic agent, for preparing a composition for treating diseases -
AN ABK10334 DNA DGENE
AB The invention describes a nucleic acid binding polypeptide (I) capable of binding to one or more of telomeric, G-quadruplex, or G-quartet nucleic acid as an inhibitor of enzymatic activity, for the preparation of a pharmaceutical composition for the treatment of a disease, or as a cytotoxic agent. (I) is useful for: inhibiting an enzymatic activity; preventing replication of a retrovirus e.g. for treating human immunodeficiency virus (HIV) infection or acquired immunodeficiency syndrome (AIDS); treating hyperproliferative disease, such as cancer; assaying a telomerase activity by providing a nucleic acid substrate for telomerase; determining the length of a telomere; discriminating between duplex and quadruplex nucleic acid; detecting telomeric structures in a system; and identifying a molecule capable of binding to a telomeric, G-quadruplex, or G-quartet structure in a nucleic acid. (I) is useful for the preparation of a pharmaceutical composition for the treatment of a disease, as a cytotoxic agent, and for killing a cell, preferably by inducing apoptosis. The assay for detecting telomerase activity using (I) is convenient, rapid, easily automated with liquid handling robotics and avoids the need to use radioactivity. This sequence represents an oligonucleotide used in the **production** of a **zinc finger** phage display library, described in the **method** of the invention.
ACCESSION NUMBER: ABK10334 DNA DGENE
TITLE: Use of a nucleic acid binding polypeptide capable of binding to telomeric, G-quadruplex, or G-quartet nucleic acid as an enzymatic activity inhibitor or cytotoxic agent, for preparing a composition for treating diseases -
INVENTOR: Choo Y; Isalan M; Liu X; Patel S; Balasubramanian S
PATENT ASSIGNEE: (SANG-N)SANGAMO BIOSCIENCES INC.
(UYCA-N) UNIV CAMBRIDGE TECH SERVICES LTD.
PATENT INFO: WO 2002004488 A2 20020117 147p
APPLICATION INFO: WO 2001-GB3130 20010712
PRIORITY INFO: US 2000-614679 20000712
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: 2002-216951 [27]

L5 ANSWER 82 OF 84 DGENE (C) 2002 THOMSON DERWENT
TI Use of a nucleic acid binding polypeptide capable of binding to telomeric, G-quadruplex, or G-quartet nucleic acid as an enzymatic activity inhibitor or cytotoxic agent, for preparing a composition for treating diseases -
AN ABK10333 DNA DGENE
AB The invention describes a nucleic acid binding polypeptide (I) capable of binding to one or more of telomeric, G-quadruplex, or G-quartet nucleic acid as an inhibitor of enzymatic activity, for the preparation of a pharmaceutical composition for the treatment of a disease, or as a cytotoxic agent. (I) is useful for: inhibiting an enzymatic activity; preventing replication of a retrovirus e.g. for treating human

immunodeficiency virus (HIV) infection or acquired immunodeficiency syndrome (AIDS); treating hyperproliferative disease, such as cancer; assaying a telomerase activity by providing a nucleic acid substrate for telomerase; determining the length of a telomere; discriminating between duplex and quadruplex nucleic acid; detecting telomeric structures in a system; and identifying a molecule capable of binding to a telomeric, G-quadruplex, or G-quartet structure in a nucleic acid. (I) is useful for the preparation of a pharmaceutical composition for the treatment of a disease, as a cytotoxic agent, and for killing a cell, preferably by inducing apoptosis. The assay for detecting telomerase activity using (I) is convenient, rapid, easily automated with liquid handling robotics and avoids the need to use radioactivity. This sequence represents an oligonucleotide used in the **production** of a **zinc finger** phage display library, described in the **method** of the invention.

ACCESSION NUMBER: ABK10333 DNA DGENE
TITLE: Use of a nucleic acid binding polypeptide capable of binding to telomeric, G-quadruplex, or G-quartet nucleic acid as an enzymatic activity inhibitor or cytotoxic agent, for preparing a composition for treating diseases -
INVENTOR: Choo Y; Isalan M; Liu X; Patel S; Balasubramanian S
PATENT ASSIGNEE: (SANG-N) SANGAMO BIOSCIENCES INC.
(UYCA-N) UNIV CAMBRIDGE TECH SERVICES LTD.
PATENT INFO: WO 2002004488 A2 20020117 147p
APPLICATION INFO: WO 2001-GB3130 20010712
PRIORITY INFO: US 2000-614679 20000712
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: 2002-216951 [27]

L5 ANSWER 83 OF 84 DGENE (C) 2002 THOMSON DERWENT
TI Use of a nucleic acid binding polypeptide capable of binding to telomeric, G-quadruplex, or G-quartet nucleic acid as an enzymatic activity inhibitor or cytotoxic agent, for preparing a composition for treating diseases -
AN ABK10332 DNA DGENE
AB The invention describes a nucleic acid binding polypeptide (I) capable of binding to one or more of telomeric, G-quadruplex, or G-quartet nucleic acid as an inhibitor of enzymatic activity, for the preparation of a pharmaceutical composition for the treatment of a disease, or as a cytotoxic agent. (I) is useful for: inhibiting an enzymatic activity; preventing replication of a retrovirus e.g. for treating human immunodeficiency virus (HIV) infection or acquired immunodeficiency syndrome (AIDS); treating hyperproliferative disease, such as cancer; assaying a telomerase activity by providing a nucleic acid substrate for telomerase; determining the length of a telomere; discriminating between duplex and quadruplex nucleic acid; detecting telomeric structures in a system; and identifying a molecule capable of binding to a telomeric, G-quadruplex, or G-quartet structure in a nucleic acid. (I) is useful for the preparation of a pharmaceutical composition for the treatment of a disease, as a cytotoxic agent, and for killing a cell, preferably by inducing apoptosis. The assay for detecting telomerase activity using (I) is convenient, rapid, easily automated with liquid handling robotics and avoids the need to use radioactivity. This sequence represents an oligonucleotide used in the **production** of a **zinc finger** phage display library, described in the **method** of the invention.

ACCESSION NUMBER: ABK10332 DNA DGENE
TITLE: Use of a nucleic acid binding polypeptide capable of binding to telomeric, G-quadruplex, or G-quartet nucleic acid as an enzymatic activity inhibitor or cytotoxic agent, for preparing a composition for treating diseases -

INVENTOR: Choo Y; Isalan M; Liu X; Patel S; Balasubramanian S
PATENT ASSIGNEE: (SANG-N) SANGAMO BIOSCIENCES INC.
(UYCA-N) UNIV CAMBRIDGE TECH SERVICES LTD.
PATENT INFO: WO 2002004488 A2 20020117 147p
APPLICATION INFO: WO 2001-GB3130 20010712
PRIORITY INFO: US 2000-614679 20000712
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: 2002-216951 [27]

L5 ANSWER 84 OF 84 WPIDS (C) 2002 THOMSON DERWENT
TI New library of nucleic acid binding **zinc finger** polypeptide(s) - each polypeptide comprising more than one **zinc finger** which is partially randomised, useful for detecting a target nucleic acid sequence.
AN 1999-024577 [02] WPIDS
CR 1999-024578 [02]; 1999-045309 [04]
AB WO 9853057 A UPAB: 20020215
A **zinc finger** polypeptide library (I) in which each polypeptide comprises more than one **zinc finger** which has been at least partially randomised is new.

Also claimed are: (1) a set (II) of **zinc finger** polypeptide libraries which encode overlapping **zinc finger** polypeptides which may be assembled after selection to form a multifinger **zinc finger** polypeptide; and (2) a **method** of preparing a library of nucleic acid (NA) binding proteins of the Cys2-His2 **zinc finger** class capable of binding to a target NA sequence.

USE - The **method** of (2) is useful for specifically engineering **zinc finger** proteins which can bind to particular nucleic acid targets. The resulting proteins can be used for determining the presence of a target nucleic acid (claimed). The proteins of the invention can be used in the manufacture of chimeric restriction enzymes, in which a NA cleaving domain is fused to a NA binding domain comprising a **zinc finger**. Fusion proteins comprising a binding protein and an integrase, e.g. viral integrase, can be used to target NA sequences in vivo. In gene therapy applications, the **method** may be targeted to the delivery of functional genes into defective genes, or the delivery of nonsense NA in order to disrupt undesired NA. Genes may also be delivered to known, repetitive stretches of nucleic acid, e.g. centromeres, together with an activating sequence such as an LCR. NA binding proteins can be specifically used to knock-out cells having mutant proteins, e.g. mutant ras. They can also be used to modulate the action of transcription factors, e.g. the activity of HIV tat may be reduced by binding proteins specific for HIV TAR. The new binding proteins may also be coupled to toxic molecules, e.g. nucleases, which are capable of selectively destroying cells which comprise a mutation in their endogenous nucleic acid. The products can be used in the treatment of infections.

ADVANTAGE - The invention provides a code of amino acid position bias which permits the selection of the library against any target nucleic acid sequence, and the **production** of a specific **nucleic acid binding protein**. Synergistic interactions between adjacent zinc fingers are taken into account, allowing the selection of any desired binding site. The invention allows the definition of every residue in a **zinc finger** nucleic acid binding motif which will bind specifically to a given nucleic acid quadruplet. When a marker protein is co-expressed with the binding protein, the requirement for gel electrophoresis is obviated, and so opens the way for automated nucleic acid diagnosis.

Dwg.0/6
ACCESSION NUMBER: 1999-024577 [02] WPIDS

CROSS REFERENCE: 1999-024578 [02]; 1999-045309 [04]
DOC. NO. CPI: C1999-007688
TITLE: New library of nucleic acid binding **zinc finger** polypeptide(s) - each polypeptide comprising more than one **zinc finger** which is partially randomised, useful for detecting a target nucleic acid sequence.
DERWENT CLASS: B04 D16
INVENTOR(S): CHOO, Y; ISALAN, M; KLUG, A
PATENT ASSIGNEE(S): (MEDI-N) MEDICAL RES COUNCIL; (GEND-N) GENDAQ LTD
COUNTRY COUNT: 83
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 9853057	A1	19981126	(199902)*	EN	56
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SZ UG ZW					
W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH GM GW HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZW					
AU 9875422	A	19981211	(199917)		
EP 983349	A1	20000308	(200017)	EN	
R: AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE					
AU 737756	B	20010830	(200155)		
JP 2002502238	W	20020122	(200211)		56

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 9853057	A1	WO 1998-GB1510	19980526
AU 9875422	A	AU 1998-75422	19980526
EP 983349	A1	EP 1998-922963	19980526
		WO 1998-GB1510	19980526
AU 737756	B	AU 1998-75422	19980526
JP 2002502238	W	JP 1998-550153	19980526
		WO 1998-GB1510	19980526

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 9875422	A	Based on	WO 9853057
EP 983349	A1	Based on	WO 9853057
AU 737756	B	Previous Publ.	AU 9875422
		Based on	WO 9853057
JP 2002502238	W	Based on	WO 9853057

PRIORITY APPLN. INFO: GB 1997-10809 19970523

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Refine Search

Search Results -

Terms	Documents
L6 and cys2-his2	7

Database:
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Derwent World Patents Index
IBM Technical Disclosure Bulletins

Search: L8

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L4	L3 and l1	1229	L4
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☐ 1. Document ID: US 6607882 B1

L8: Entry 1 of 7 File: USPT Aug 19, 2003

US-PAT-NO: 6607882
DOCUMENT-IDENTIFIER: US 6607882 B1

TITLE: Regulation of endogenous gene expression in cells using zinc finger proteins

DATE-ISSUED: August 19, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Cox, III; George N.	Louisville	CO		
Case; Casey C.	San Mateo	CA		
Eisenberg; Stephen P.	Boulder	CO		
Jarvis; Eric E.	Boulder	CO		
Spratt; Sharon K.	Vacaville	CA		

US-CL-CURRENT: 435/6; 435/320.1, 435/455, 435/468, 536/23.1, 536/23.4, 536/24.1

Full | Title | Citation | Front | Review | Classification | Date | Reference | [illegible] | Claims | KWIC | Draw De

☐ 2. Document ID: US 6599692 B1

L8: Entry 2 of 7 File: USPT Jul 29, 2003

US-PAT-NO: 6599692
DOCUMENT-IDENTIFIER: US 6599692 B1

TITLE: Functional genomics using zinc finger proteins

DATE-ISSUED: July 29, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Case; Casey C.	San Mateo	CA		
Zhang; Lei	San Francisco	CA		

US-CL-CURRENT: 435/4; 435/6, 536/23.1

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Full | Title | Citation | Front | Review | Classification | Date | Reference | ... | Claims | KWMC | Draw De

☐ 3. Document ID: US 6534261 B1

L8: Entry 3 of 7 File: USPT Mar 18, 2003

US-PAT-NO: 6534261
DOCUMENT-IDENTIFIER: US 6534261 B1

TITLE: Regulation of endogenous gene expression in cells using zinc finger proteins

DATE-ISSUED: March 18, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Cox, III; George Norbert	Louisville	CO		
Case; Casey Christopher	San Mateo	CA		
Eisenberg; Stephen P.	Boulder	CO		
Jarvis; Eric Edward	Boulder	CO		
Spratt; Sharon Kaye	Vacaville	CA		

US-CL-CURRENT: 435/6; 435/29, 536/23.5, 536/24.1

Full | Title | Citation | Front | Review | Classification | Date | Reference | ... | Claims | KWMC | Draw De

☐ 4. Document ID: US 6503717 B2

L8: Entry 4 of 7 File: USPT Jan 7, 2003

US-PAT-NO: 6503717
DOCUMENT-IDENTIFIER: US 6503717 B2

TITLE: Methods of using randomized libraries of zinc finger proteins for the identification of gene function

DATE-ISSUED: January 7, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Case; Casey C.	San Mateo	CA		
Liu; Qiang	Foster City	CA		
Rebar; Edward J.	El Cerrito	CA		
Wolffe; Alan P.	Orinda	CA		

US-CL-CURRENT: 435/6; 435/320.1, 435/455, 536/23.5

Full | Title | Citation | Front | Review | Classification | Date | Reference | ... | Claims | KWMC | Draw De

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☐ 5. Document ID: US 6492117 B1

L8: Entry 5 of 7 File: USPT Dec 10, 2002

US-PAT-NO: 6492117
DOCUMENT-IDENTIFIER: US 6492117 B1

TITLE: Zinc finger polypeptides capable of binding DNA quadruplexes

DATE-ISSUED: December 10, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Choo; Yen	London			GB
Isalan; Mark	London			GB
Patel; Sachin D.	Mumbai			IN
Balasubramanian; Shhankar	Cambridge			GB
Liu; Xiaohai	Cambridge			GB

US-CL-CURRENT: 435/6; 436/501, 530/300, 530/324

Full | Title | Citation | Front | Review | Classification | Date | Reference | ... | Claims | KWMC | Draw De

☐ 6. Document ID: US 6453242 B1

L8: Entry 6 of 7 File: USPT Sep 17, 2002

US-PAT-NO: 6453242
DOCUMENT-IDENTIFIER: US 6453242 B1

TITLE: Selection of sites for targeting by zinc finger proteins and methods of designing zinc finger proteins to bind to preselected sites

DATE-ISSUED: September 17, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Eisenberg; Stephen P.	Boulder	CO		
Case; Casey C.	San Mateo	CA		
Cox, III; George N.	Louisville	CO		
Jamieson; Andrew	San Francisco	CA		
Rebar; Edward J.	Berkeley	CA		

US-CL-CURRENT: 702/19; 435/6, 702/20, 702/21

Full | Title | Citation | Front | Review | Classification | Date | Reference | ... | Claims | KWMC | Draw De

☐ 7. Document ID: US 6410248 B1

L8: Entry 7 of 7 File: USPT Jun 25, 2002

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US-PAT-NO: 6410248
DOCUMENT-IDENTIFIER: US 6410248 B1

TITLE: General strategy for selecting high-affinity zinc finger proteins for diverse DNA target sites

DATE-ISSUED: June 25, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Greisman; Harvey A.	Brookline	MA		
Pabo; Carl O.	Newton	MA		

US-CL-CURRENT: 435/7.2; 435/4, 435/5, 435/6, 435/69.1, 435/DIG.14, 435/DIG.15, 435/DIG.2, 435/DIG.3, 435/DIG.4, 436/501

Terms	Documents
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L8: Entry 1 of 7 File: USPT Aug 19, 2003

DOCUMENT-IDENTIFIER: US 6607882 B1
TITLE: Regulation of endogenous gene expression in cells using zinc finger proteins

Abstract Text (1):
The present invention provides methods for modulating expression of endogenous cellular genes using recombinant zinc finger proteins.

Brief Summary Text (2):
The present invention provides methods for regulating gene, expression of endogenous genes using recombinant zinc finger proteins.

Brief Summary Text (4):
Many pathophysiological processes are the result of aberrant gene expression. Examples include the inappropriate activation of proinflammatory cytokines in rheumatoid arthritis, under-expression of the hepatic LDL receptor in hypercholesteremia, over-expression of proangiogenic factors, and under-expression of antiangiogenic factors in solid tumor growth. If therapeutic methods for control of gene expression existed, many of these pathologies could be more optimally treated. In another example, developmentally silent or otherwise inactive genes could be activated in order to treat a particular disease state. Inactive genes are repressed via several mechanisms, including chromatin structure, specific cis-acting repressors, and DNA methylation (Travers, Cell 96:311(1996); Beato & Eisfeld, Nucleic Acids Res. 25:3559(1997); Wolffe et al., PNAS 96:5894(1999)). Examples of the therapeutic benefit for expression of such genes include activation of developmentally silent fetal hemoglobin genes to treat sickle cell disease and the activation of eutrophin to treat muscular dystrophy. In addition, pathogenic organisms such as viruses, bacteria, fungi, and protozoa could be controlled by altering gene expression. There is thus a clear unmet need for therapeutic approaches that act through sequence-specific regulation of diseaserelated genes.

Brief Summary Text (6):
An additional use of tools permitting the manipulation of gene expression is in the production of commercially useful biological products. Cell lines, transgenic animals and transgenic plants could be engineered to over-express a useful protein product. The production of erythropoietin by such an engineered cell line serves as an example. Likewise, production from metabolic pathways might be altered or improved by the selective up or down-regulation of a gene encoding a crucial enzyme. An example of this is the production of plants with altered levels of fatty acid saturation.

Brief Summary Text (8):
Gene expression is normally controlled through alterations in the function of sequence specific DNA binding proteins called transcription factors. These bind in the general proximity (although occasionally at great distances) of the point of transcription initiation of a gene. They act to influence the efficiency of formation or function of a transcription initiation complex at the promoter. Transcription factors can act in a positive fashion (transactivation) or in a negative fashion (transrepression).

Brief Summary Text (9):

Transcription factor function can be constitutive (always "on") or conditional. Conditional function can be imparted on a transcription factor by a variety of means, but the majority of these regulatory mechanisms depend of the sequestering of the factor in the cytoplasm and the inducible release and subsequent nuclear translocation, DNA binding and transactivation (or repression). Examples of transcription factors that function this way include progesterone receptors, sterol response element binding proteins (SREBPs) and NF-kappa B. There are examples of transcription factors that respond to phosphorylation or small molecule ligands by altering their ability to bind their cognate DNA recognition sequence (Hou et al., Science 256:1701 (1994); Gossen & Bujard, PNAS 89:5547 (1992); Oligino et al., Gene Ther. 5:491-496 (1998); Wang et al., Gene Ther. 4:432-441 (1997); Neering et al., Blood 88:1147-1155 (1996); and Rendahl et al., Nat. Biotechnol. 16:757-761 (1998)). This mechanism is common in prokaryotes but somewhat less common in eukaryotes.

Brief Summary Text (10):
Zinc finger proteins ("ZFPs") are proteins that can bind to DNA in a sequence-specific manner. Zinc fingers were first identified in the transcription factor TFIIIA from the oocytes of the African clawed toad, Xenopus laevis. ZFPs are widespread in eukaryotic cells. An exemplary motif characterizing one class of these proteins (C.sub.2 H.sub.2 class) is -Cys-(X).sub.2-4 -Cys-(X).sub.12 -His-(X).sub.3-5 -His (SEQ ID NO:1) (where X is any amino acid). A single finger domain is about 30 amino acids in length and several structural studies have demonstrated that it contains an alpha helix containing the two invariant histidine residues co-ordinated through zinc with the two cysteines of a single beta turn. To date, over 10,000 zinc finger sequences have been identified in several thousand known or putative transcription factors. ZFPs are involved not only in DNA-recognition, but also in RNA binding and protein-protein binding. Current estimates are that this class of molecules will constitute about 2% of all human genes.

Brief Summary Text (11):
The X-ray crystal structure of Zif268, a three-finger domain from a murine transcription factor, has been solved in complex with its cognate DNA-sequence and shows that each finger can be superimposed on the next by a periodic rotation and translation of the finger along the main DNA axis. The structure suggests that each finger interacts independently with DNA over 3 base-pair intervals, with side-chains at positions -1, 2 , 3 and 6 on each recognition helix making contacts with respective DNA triplet sub-site. The amino terminus of Zif268 is situated at the 3' end of its DNA recognition subsite. Some zinc fingers can bind to a fourth base in a target segment. The fourth base is on the opposite strand from the other three bases recognized by zinc finger and complementary to the base immediately 3' of the three base subsite.

Brief Summary Text (12):
The structure of the Zif268-DNA complex also suggested that the DNA sequence specificity of a ZFP might be altered by making amino acid substitutions at the four helix positions (-1, 2, 3 and 6) on a zinc finger recognition helix. Phage display experiments using zinc finger combinatorial libraries to test this observation were published in a series of papers in 1994 (Rebar et al., Science 263:671-673 (1994); Jamieson et al., Biochemistry 33:5689-5695 (1994); Choo et al., PNAS 91:11163-11167 (1994)). Combinatorial libraries were constructed with randomized side-chains in either the first or middle finger of Zif268 and then isolated with an altered Zif268 binding site in which the appropriate DNA sub-site was replaced by an altered DNA triplet. Correlation between the nature of introduced mutations and the resulting alteration in binding specificity gave rise to a partial set of substitution rules for rational design of ZFPs with altered binding specificity.

Brief Summary Text (13):
Greisman & Pabo, Science 275:657-661 (1997) discuss an elaboration of a phage display method in which each finger of a zinc finger protein is successively

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subjected to randomization and selection. This paper reported selection of ZFPs for a nuclear hormone response element, a p53 target site and a TATA box sequence.

Brief Summary Text (15):
For example, Pomerantz et al., Science 267:93-96 (1995) report an attempt to design a novel DNA binding protein by fusing two fingers from Zif268 with a homeodomain from Oct-1. The hybrid protein was then fiused with either a transcriptional activator or repressor domain for expression as a chimeric protein. The chimeric protein was reported to bind a target site representing a hybrid of the subsites of its two components. The authors then constructed a reporter vector containing a luciferase gene operably linked to a promoter and a hybrid site for the chimeric DNA binding protein in proximity to the promoter. The authors reported that their chimeric DNA binding protein could activate or repress expression of the luciferase gene.

Brief Summary Text (16):
Liu et al., PNAS 94:5525-5530 (1997) report forming a composite ZFP by using a peptide spacer to link two component ZFPs, each having three fingers. The composite protein was then further linked to transcriptional activation or repression domains. It was reported that the resulting chimeric protein bound to a target site formed from the target segments bound by the two component ZFPs. It was further reported that the chimeric ZFP could activate or repress transcription of a reporter gene when its target site was inserted into a reporter plasmid in proximity to a promoter operably linked to the reporter.

Brief Summary Text (17):
Beerli et al., PNAS 95:14628-14633 (1998) report construction of a chimeric six finger ZFP fused to either a KRAB, ERD, or SID transcriptional repressor domain, or the VP16 or VP64 transcriptional activation domain. This chimeric ZFP was designed to recognize an 18 bp target site in the 5' untranslated region of the human erbB-2 gene. Using this construct, the authors of this study report both activation and repression of a transiently expressed reporter luciferase construct linked to the erbB-2 promoter.

Brief Summary Text (18):
In addition, a recombinant ZFP was reported to repress expression of an integrated plasmid construct encoding a bcr-abl oncogene (Choo et al., Nature 372:642-645 (1994)). The target segment to which the ZFPs bound was a nine base sequence GCA GAA GCC chosen to overlap the junction created by a specific oncogenic translocation fusing the genes encoding bcr and abl. The intention was that a ZFP specific to this target site would bind to the oncogene without binding to abl or bcr component genes. The authors used phage display to select a variant ZFP that bound to this target segment. The variant ZFP thus isolated was then reported to repress expression of a stably transfected bcr-abl construct in a cell line.

Brief Summary Text (22):
In one aspect, the present invention provides a method of inhibiting expression of an endogenous cellular gene in a cell, the method comprising the step of: contacting a first target site in the endogenous cellular gene with a first zinc finger protein, wherein the K.sub.d of the zinc finger protein is less than about 25 nM; thereby inhibiting expression of the endogenous cellular gene by at least about 20%.

Brief Summary Text (23):
In another aspect, the present invention provides a method of inhibiting expression of an endogenous cellular gene in a cell, the method comprising the step of: contacting a target site in the endogenous cellular gene with a fusion zinc finger protein comprising six fingers and a regulatory domain, wherein the K.sub.d of the zinc finger protein is less than about 25 nM; thereby inhibiting expression of the endogenous cellular gene by at least about 20%.

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Brief Summary Text (25):
In another aspect, the present invention provides a method of activating expression of an endogenous cellular gene, the method comprising the step of: contacting a first target site in the endogenous cellular gene with a first zinc finger protein, wherein the K.sub.d of the zinc finger protein is less than about 25 nM; thereby activating expression of the endogenous cellular gene to at least about 150%.

Brief Summary Text (26):
In another aspect, the present invention provides a method of activating expression of an endogenous cellular gene, the method comprising the step of: contacting a target site in the endogenous cellular gene with a fusion zinc finger protein comprising six fingers and a regulatory domain, wherein the K.sub.d of the zinc finger protein is less than about 25 nM; thereby activating expression of the endogenous cellular gene to at least about 150%.

Brief Summary Text (28):
In another aspect, the present invention provides a method of modulating expression of an endogenous cellular-gene in a cell, the method comprising the step of: contacting a first target site in the endogenous cellular gene with a first zinc finger protein; thereby modulating expression of the endogenous cellular gene.

Brief Summary Text (29):
In one embodiment, the zinc finger protein has two, three, four, five, or six fingers.

Brief Summary Text (30):
In another aspect, the present invention provides a method of modulating expression of an endogenous cellular gene in a cell, the method comprising the step of: contacting a target site in the endogenous cellular gene with a fusion zinc finger protein comprising six fingers and a regulatory domain; thereby modulating expression of the endogenous cellular gene.

Brief Summary Text (31):
In one embodiment, the step of contacting further comprises contacting a second target site in the endogenous cellular gene with a second zinc finger protein. In another embodiment, the first and second target sites are adjacent. In another embodiment, the first and second zinc finger proteins are covalently linked. In another embodiment, the first zinc finger protein is a fusion protein comprising a regulatory domain. In another embodiment, the first zinc finger protein is a fusion protein comprising at least two regulatory domains. In another embodiment, the first and second zinc finger proteins are fusion proteins, each comprising a regulatory domain. In another embodiment, the first and second zinc finger protein are fusion proteins, each comprising at least two regulatory domains.

Brief Summary Text (32):
In one embodiment, the endogenous cellular gene is a selected from the group consisting of VEGF, ER.alpha., IGF-I, c-myc, c-myb, ICAM, Her2/Neu, FAD2-1, EPO, GM-CSF, GDNF, and LDL-R. In another embodiment, the endogenous cellular gene is a developmentally silent or otherwise inactive gene, e.g., EPO, GATA, interleukin family proteins, GM-CSF, MyoD, eutrophin, and fetal hemoglobins gamma and delta. In another embodiment, the regulatory domain is selected from the group consisting of a transcriptional repressor, a transcriptional activator, an endonuclease, a methyl transferase, a histone acetyltransferase, and a histone deacetylase.

Brief Summary Text (34):
In one embodiment, the method further comprises the step of first administering to the cell a delivery vehicle comprising the zinc finger protein, wherein the delivery vehicle comprises a liposome or a membrane translocation polypeptide.

Brief Summary Text (35):
In one embodiment, the zinc finger protein is encoded by a zinc finger protein nucleic acid operably linked to a promoter, and the method further comprises the step of first administering the nucleic acid to the cell in a lipid:nucleic acid complex or as naked nucleic acid. In another embodiment, the zinc finger protein is encoded by an expression vector comprising a zinc finger protein nucleic acid operably linked to a promoter, and the method further comprises the step of first administering the expression vector to the cell. In another embodiment, the expression vector is a viral expression vector. In another embodiment, the expression vector is a retroviral expression vector, an adenoviral expression vector, a DNA plasmid expression vector, or an AAV expression vector.

Brief Summary Text (36):
In one the zinc finger protein is encoded by a nucleic acid operably linked to an inducible promoter. In another embodiment, the zinc finger protein is encoded by a nucleic acid operably linked to a weak promoter.

Brief Summary Text (37):
In one embodiment, the cell comprises less than about 1.5.times.10.sup.6 copies of the zinc finger protein.

Brief Summary Text (39):
In another embodiment, the zinc finger protein comprises an SP-1 backbone. In one embodiment, the zinc finger protein comprises a regulatory domain and is humanized.

Drawing Description Text (4):
FIG. 3. Typical EMSA experiment with MBP fused ZFP. MBP-VEGF1 protein was bound to labeled duplex DNA as described in the text. A three-fold protein dilution series was carried out; each point represents the percent shifted at that particular protein concentration plotted on a semi-log graph. Quantitation was by phosphorimager. In this case, the protein concentration yielding 50% of maximum shift (the apparent K.sub.d) was 2 nM.

Drawing Description Text (5):
FIG. 4, panels A and B depict results of off-rate experiments comparing VEGF1 to VEGF3a/1. Protein-DNA complexes were pre-formed and incubated with a 1000-fold excess of unlabeled oligonucleotide. Samples were electrophoresed at various times and the amount of shifted product was measured by phosphorimager. Curve fitting was used to calculate the indicated complex half-lives.

Drawing Description Text (7):
FIG. 6. Co-transfection data showing repression of luciferase reporter activity via VEGF-KRAB protein expression. Error bars show the standard deviation of triplicate transfections. pGL3-C (reporter vector control); pVFR1-4x (VEGF reporter plasmid); VEGF1 (VEGF1-KRAB); VEGF3a (VEGF3a-KRAB); VEGF3a/1 (VEGF3a/1-KRAB).

Drawing Description Text (8):
FIG. 7. Co-transfection data showing activation of luciferase reporter activity via VEGF-VP16 protein expression. Error bars show the standard deviation of triplicate transfections. pGL3-P (reporter with no VEGF target); pcDNA (empty effector vector control); pVFR3-4x (VEGF reporter plasmid); VEGF1 (VEGF1-VP16); VEGF3a (VEGF3a-VP 16); VEGF3a/1 (VEGF3a/1-VP 16).

Detailed Description Text (3):
The present application demonstrates for the first time that ZFPs can be used to regulate expression of an endogenous cellular gene that is present in its native chromatin environment. The present invention thus provides zinc finger DNA binding proteins that have been engineered to specifically recognize, with high efficacy, endogenous cellular genes. The experiments described herein demonstrate that a 3

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finger ZFP with a target site affinity of less than about 10 nM (VEGF1) can be used to effectively activate or repress activity of an endogenous gene. Furthermore, a 6 finger ZFP (VEGF3a/1) was also shown to effectively repress activity of an endogenous gene. Finally, three finger ZFP can be used to activate endogenous EPO, a developmentally inactive gene. Preferably, the ZFPs of the invention exhibit high affinity for their target sites, with K.sub.d s of less than about 100 nM, preferably less than about 50 nM, most preferably less than about 25 nM or lower.

Detailed Description Text (6):
As described herein, ZFPs can be designed to recognize any suitable target site, for regulation of expression of any endogenous gene of choice. Examples of endogenous genes suitable for regulation include VEGF, CCR5, ER.alpha., Her2/Neu, Tat, Rev, HBV C, S, X, and P, LDL-R, PEPCK, CYP7, Fibrinogen, ApoB, Apo E, Apo(a), renin, NF-.kappa.B, I-.kappa.B, TNF-.alpha., FAS ligand, amyloid precursor protein, atrial naturetic factor, ob-leptin, ucp-1, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-12, G-CSF, GM-CSF, Epo, PDGF, PAF, p53, Rb, fetal hemoglobin, dystrophin, eutrophin, GDNF, NGF, IGF-1, VEGF receptors flt and flk, topoisomerase, telomerase, bcl-2, cyclins, angiostatin, IGF, ICAM-1, STATS, c-myc, c-myb, TH, PTI-1, polygalacturonase, EPSP synthase, FAD2-1, delta-12 desaturase, delta-9 desaturase, delta-15 desaturase, acetyl-CoA carboxylase, acyl-ACP-thioesterase, ADP-glucose pyrophosphorylase, starch synthase, cellulose synthase, sucrose synthase, senescence-associated genes, heavy metal chelators, fatty acid hydroperoxide lyase, viral genes, protozoal genes, flngal genes, and bacterial genes. In general, suitable genes to be regulated include cytokines, lymphokines, growth factors, mitogenic factors, chemotactic factors, onco-active factors, receptors, potassium channels, G-proteins, signal transduction molecules, and other disease-related genes. Preferred developmentally inactive genes include EPO, GATA, interleukin family proteins, GM-CSF, MyoD, eutrophin, and fetal hemoglobins gamma and delta.

Detailed Description Text (7):
A general theme in transcription factor function is that simple binding and sufficient proximity to the promoter are all that is generally needed. Exact positioning relative to the promoter, orientation, and within limits, distance, do not matter greatly for expression modulation by a ZFP. This feature allows considerable flexibility in choosing sites for constructing artificial transcription factors. The target site recognized by the ZFP therefore can be any suitable site in the target gene that will allow activation or repression of gene expression by a ZFP, optionally linked to a regulatory domain. Preferred target sites include regions adjacent to, downstream, or upstream of the transcription start site. In addition, target sites can also be located in enhancer regions, repressor sites, RNA polymerase pause sites, and specific regulatory sites (e.g., SP-1 sites, hypoxia response elements, nuclear receptor recognition elements, p53 binding sites), sites in the cDNA encoding region or in an expressed sequence tag (EST) coding region. As described below, typically each finger recognizes 2-4 base pairs, with a two finger ZFP binding to a 4 to 7 bp target site, a three finger ZFP binding to a 6 to 10 base pair site, and a six finger ZFP binding to two adjacent target sites, each target site having from 6-10 base pairs.

Detailed Description Text (8):
As described herein, two ZFPs can be administered to a cell, recognizing either the same target endogenous cellular gene, or different target endogenous cellular gene. The first ZFP optionally is associated with the second ZFP, either covalently or non-covalently. Recognition of adjacent target sites by either associated or individual ZFPs can be used to produce cooperative binding of the ZFPs, resulting in an affinity that is greater than the affinity of the ZFPs when individually bound to their target site.

Detailed Description Text (9):
In one embodiment, two ZFPs are produced as a fusion protein linked by an amino acid linker, and the resulting six finger ZFP recognizes an approximately 18 base

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pair target site (see, e.g., Liu et al., PNAS 94:5525-5530 (1997)). An 18 base pair target site is expected to provide specificihuty in the human genome, as a target site of that size should occur only once in every 3.times.10.sup.10 base pairs, and the size of the human genome is 3.5.times.10.sup.9 base pairs (see, e.g., Liu et al., PNAS 94:5525-5530 (1997)). In another embodiment, the ZFPs are non-covalently associated, through a leucine zipper, a STAT protein N-terminal domain, or the FK506 binding protein (see, e.g., O'Shea, Science 254: 539 (1991), Barahmand-Pour et al., Curr. Top. Microbiol. Immunol. 211:121-128 (1996); Klemm et al., Annu. Rev. Immunol. 16:569-592 (1998); Ho et al., Nature 382:822-826 (1996)).

Detailed Description Text (14):
The term "zinc finger protein", or "ZFP" refers to a protein having DNA binding domains that are stabilized by zinc. The individual DNA binding domains are typically referred to as "fingers" A zinc finger protein has least one finger, typically two fingers, three fingers, four fingers, five fingers, or six fingers or more. Each-finger binds from two to four base pairs of DNA, typically three or four base pairs of DNA. A zinc finger protein binds to a nucleic acid sequence called a target site or target segment. Each finger typically comprises an approximately 30 amino acid, zinc-coordinating, DNA-binding subdomain. An exemplary motif characterizing one class of these proteins (Cys.sub.2 His.sub.2 class) is -Cys-(X).sub.2-4 -Cys-(X).sub.12 -His-(X).sub.3-5 -His (SEQ ID NO:1) (where X is any amino acid). Studies have demonstrated that a single zinc finger of this class consists of an alpha helix containing the two invariant histidine residues co-ordinated with zinc along with the two cysteine residues of a single beta turn (see, e.g., Berg & Shi, Science 271:1081-1085 (1996)).

Detailed Description Text (15):
A "target site" is the nucleic acid sequence recognized by a zinc finger protein. A single target site typically has about four to about ten or more base pairs. Typically, a two-fingered zinc finger protein recognizes a four to seven base pair target site, a three-fingered zinc finger protein recognizes a six to ten base pair target site, a six fingered zinc finger protein recognizes two adjacent nine to ten base pair target sites, and so on for proteins with more than six fingers. The target site is in any position that allows regulation of gene expression, e.g., adjacent to, up- or downstream of the transcription initiation site; proximal to an enhancer or other transcriptional regulation element such as a repressor (e.g., SP-1 binding sites, hypoxia response elements, nuclear receptor recognition elements, p53 binding sites, etc.), RNA polymerase pause sites; and intron/exon boundaries. The term "adjacent target sites" refers to non-overlapping target sites that are separated by zero to about 5 base pairs.

Detailed Description Text (16):
"K.sub.d " refers to the dissociation constant for the compound, i.e., the concentration of a compound (e.g., a zinc finger protein) that gives half maximal binding of the compound to its target (i.e., half of the compound molecules are bound to the target) under given conditions (i.e., when [target]<<K.sub.d), as measured using a given assay system (see, e.g., U.S. Pat. No. 5,789,538). The assay system used to measure the K.sub.d should be chosen so that it gives the most accurate measure of the actual K.sub.d of the ZFP. Any assay system can be used, as long is it gives an accurate measurement of the actual K.sub.d of the ZFP. In one embodiment, the K.sub.d for the ZFPs of the invention is measured using an electrophoretic mobility shift assay ("EMSA"), as described in Example I and on page 14 of the present specification. Unless an adjustment is made for ZFP purity or activity, the K.sub.d calculations made using the method of Example I may result in an underestimate of the true K.sub.d of a given ZFP. Preferably, the K.sub.d of a ZFP used to modulate transcription of an endogenous cellular gene is less than about 100 nM, more preferably less than about 75 nM, more preferably less than about 50 nM, most preferably less than about 25 nM.

Detailed Description Text (19):

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A "native chromatin environment" refers to the naturally occurring, structural relationship of genomic DNA (e.g., bacterial, animal, fangal, plant, protozoal, mitochondrial, and chloroplastic) and DNA-binding proteins (e.g., histones and bacterial DNA binding protein II), which together form chromosomes. The endogenous cellular gene can be in a transcriptionally active or inactive state in the native chromatin environment.

Detailed Description Text (20):
A "developmentally silent gene" or an "inactive gene" refers to a gene whose expression is repressed or not activated, i.e., turned off, in certain cell types, during certain developmental stages of a cell type, or during certain time periods in a cell type. Examples of developmentally inactive genes include EPO, GATA, interleukin family proteins, GM-CSF, MyoD, eutrophin, and fetal hemoglobins gamma and delta.

Detailed Description Text (23):
"Humanized" refers to a non-human polypeptide sequence that has been modified to minimize immunoreactivity in humans, typically by altering the amino acid sequence to mimic existing human sequences, without substantially altering the function of the polypeptide sequence (see, e.g., Jones et al., Nature 321:522-525 (1986), and published UK patent application No. 8707252). Backbone sequences for the ZFPs are preferably be selected from existing human C.sub.2 H.sub.2 ZFPs (e.g., SP-1). Functional domains are preferably selected from existing human genes, (e.g., the activation domain from the p65 subunit of NF-.kappa.B). Where possible, the recognition helix sequences will be selected from the thousands of existing ZFP DNA recognition domains provided by sequencing the human genome. As much as possible, domains will be combined as units from the same existing proteins. All of these steps will minimize the introduction of new junctional epitopes in the chimeric ZFPs and render the engineered ZFPs less immunogenic.

Detailed Description Text (24):
"Administering" an expression vector, nucleic acid, ZFP, or a delivery vehicle to a cell comprises transducing, transfecting, electroporating, translocating, fusing, phagocytosing, shooting or ballistic methods, etc., i.e., any means by which a protein or nucleic acid can be transported across a cell membrane and preferably into the nucleus of a cell.

Detailed Description Text (25):
A "delivery vehicle" refers to a compound, e.g., a liposome, toxin, or a membrane translocation polypeptide, which is used to administer a ZFP. Delivery vehicles can also be used to administer nucleic acids encoding ZFPs, e.g., a lipid:nucleic acid complex, an expression vector, a virus, and the like.

Detailed Description Text (27):
"Activation of gene expression that prevents repression of gene expression" refers to the ability of a zinc finger protein to block or prevent binding of a repressor molecule.

Detailed Description Text (28):
"Inhibition of gene expression that prevents gene activation" refers to the ability of a zinc finger protein to block or prevent binding of an activator molecule.

Detailed Description Text (29):
Modulation can be assayed by determining any parameter that is indirectly or directly affected by the expression of the target gene. Such parameters include, e.g., changes in RNA or protein levels, changes in protein activity, changes in product levels, changes in downstream gene expression, changes in reporter gene transcription (luciferase, CAT, .beta.-galactosidase, .beta.-glucuronidase, GFP (see, e.g., Mistili & Spector, Nature Biotechnology 15:961-964 (1997)); changes in signal transduction, phosphorylation and dephosphorylation, receptor-ligand

interactions, second messenger concentrations (e.g., cGMP, cAMP, IP3, and Ca.sup.2+), cell growth, and neovascularization. These assays can be in vitro, in vivo, and ex vivo. Such functional effects can be measured by any means known to those skilled in the art, e.g., measurement of RNA or protein levels, measurement of RNA stability, identification of downstream or reporter gene expression, e.g., via chemiluminescence, fluorescence, colorimetric reactions, antibody binding, inducible markers, ligand binding assays; changes in intracellular second messengers such as cGMP and inositol triphosphate (IP3); changes in intracellular calcium levels; cytokine release, and the like.

Detailed Description Text (30):
To determine the level of gene expression modulation by a ZFP, cells contacted with ZFPs are compared to control cells, e.g., without the zinc finger protein or with a non-specific ZFP, to examine the extent of inhibition or activation. Control samples are assigned a relative gene expression activity value of 100%. Modulation/inhibition of gene expression is achieved when the gene expression activity value relative to the control is about 80%, preferably 50% (i.e., 0.5x the activity of the control), more preferably 25%, more preferably 5-0%. Modulation/activation of gene expression is achieved when the gene expression activity value relative to the control is 110% , more preferably 150% (i.e., 1.5x the activity of the control), more preferably 200-500%, more preferably 1000-2000% or more.

Detailed Description Text (31):
A "transcriptional activator" and a "transcriptional repressor" refer to proteins or effector domains of proteins that have the ability to modulate transcription, as described above. Such proteins include, e.g., transcription factors and co-factors (e.g., KRAB, MAD, ERD, SID, nuclear factor kappa B subunit p65, early growth response factor 1, and nuclear hormone receptors, VP16, VP64), endonucleases, integrases, recombinases, methyltransferases, histone acetyltransferases, histone deacetylases etc. Activators and repressors include co-activators and co-repressors (see, e.g., Utley et al., Nature 394:498-502 (1998)).

Detailed Description Text (32):
A "regulatory domain" refers to a protein or a protein domain that has transcriptional modulation activity when tethered to a DNA binding domain, i.e., a ZFP. Typically, a regulatory domain is covalently or non-covalently linked to a ZFP to effect transcription modulation. Alternatively, a ZFP can act alone, without a regulatory domain, to effect transcription modulation.

Detailed Description Text (33):
The term "heterologous" is a relative term, which when used with reference to portions of a nucleic acid indicates that the nucleic acid comprises two or more subsequences that are not found in the same relationship to each other in nature. For instance, a nucleic acid that is recombinantly produced typically has two or more sequences from unrelated genes synthetically arranged to make a new functional nucleic acid, e.g., a promoter from one source and a coding region from another source. The two nucleic acids are thus heterologous to each other in this context. When added to a cell, the recombinant nucleic acids would also be heterologous to the endogenous genes of the cell. Thus, in a chromosome, a heterologous nucleic acid would include an non-native (non-naturally occurring) nucleic acid that has integrated into the chromosome, or a non-native (non-naturally occurring) extrachromosomal nucleic acid. In contrast, a naturally translocated piece of chromosome would not be considered heterologous in the context of this patent application, as it comprises an endogenous nucleic acid sequence that is native to the mutated cell.

Detailed Description Text (34):
Similarly, a heterologous protein indicates that the protein comprises two or more subsequences that are not found in the same relationship to each other in nature

(e.g., a "fusion protein," where the two subsequences are encoded by a single nucleic acid sequence). See, e.g., Ausubel, supra, for an introduction to recombinant techniques.

Detailed Description Text (35):
The term "recombinant" when used with reference, e.g., to a cell, or nucleic acid, protein, or vector, indicates that the cell, nucleic acid, protein or vector, has been modified by the introduction of a heterologous nucleic acid or protein or the alteration of a native nucleic acid or protein, or that the cell is derived from a cell so modified. Thus, for example, recombinant cells express genes that are not found within the native (naturally occurring) form of the cell or express a second copy of a native gene that is otherwise normally or abnormally expressed, under expressed or not expressed at all.

Detailed Description Text (36):
A "promoter" is defined as an array of nucleic acid control sequences that direct transcription. As used herein, a promoter typically includes necessary nucleic acid sequences near the start site of transcription, such as, in the case of certain RNA polymerase II type promoters, a TATA element, enhancer, CCAAT box, SP-1 site, etc. As used herein, a promoter also optionally includes distal enhancer or repressor elements, which can be located as much as several thousand base pairs from the start site of transcription. The promoters often have an element that is responsive to transactivation by a DNA-binding moiety such as a polypeptide, e.g., a nuclear receptor, Gal4, the lac repressor and the like.

Detailed Description Text (39):
The term "operably linked" refers to a functional linkage between a nucleic acid expression control sequence (such as a promoter, or array of transcription factor binding sites) and a second nucleic acid sequence, wherein the expression control sequence directs transcription of the nucleic acid corresponding to the second sequence.

Detailed Description Text (40):
An "expression vector" is a nucleic acid construct, generated recombinantly or synthetically, with a series of specified nucleic acid elements that permit transcription of a particular nucleic acid in a host cell, and optionally integration or replication of the expression vector in a host cell. The expression vector can be part of a plasmid, virus, or nucleic acid fragment, of viral or non-viral origin. Typically, the expression vector includes an "expression cassette," which comprises a nucleic acid to be transcribed operably linked to a promoter. The term expression vector also encompasses naked DNA operably linked to a promoter.

Detailed Description Text (41):
By "host cell" is meant a cell that contains a ZFP or an expression vector or nucleic acid encoding a ZFP. The host cell typically supports the replication or expression of the expression vector. Host cells may be prokaryotic cells such as E. coli, or eukaryotic cells such as yeast, fungal, protozoal, higher plant, insect, or amphibian cells, or mammalian cells such as CHO, HeLa, 293, COS-1, and the like, e.g., cultured cells (in vitro), explants and primary cultures (in vitro and ex vivo), and cells in vivo.

Detailed Description Text (42):
"Nucleic acid" refers to deoxyribonucleotides or ribonucleotides and polymers thereof in either single- or double-stranded form. The term encompasses nucleic acids containing known nucleotide analogs or modified backbone residues or linkages, which are synthetic, naturally occurring, and non-naturally occurring, which have similar binding properties as the reference nucleic acid, and which are metabolized in a manner similar to the reference nucleotides. Examples of such analogs include, without limitation, phosphorothioates, phosphoramidates, methyl phosphonates, chiral-methyl phosphonates, 2-O-methyl ribonucleotides, peptide-

nucleic acids (PNAs).

Detailed Description Text (43):
Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (e.g., degenerate codon substitutions) and complementary sequences, as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer et al., Nucleic Acid Res. 19:5081 (1991); Ohtsuka et al., J. Biol. Chem. 260:2605-2608 (1985); Rossolini et al., Mol. Cell. Probes 8:91-98 (1994)). The term nucleic acid is used interchangeably with gene, cDNA, mRNA, oligonucleotide, and polynucleotide.

Detailed Description Text (44):
The terms "polypeptide," "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. The terms also apply to amino acid polymers in which one or more amino acid residues is an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers and non-naturally occurring amino acid polymer.

Detailed Description Text (45):
The term "amino acid" refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner similar to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified, e.g., hydroxyproline, .gamma.-carboxyglutamate, and O-phosphoserine. Amino acid analogs refers to compounds that have the same basic chemical structure as a naturally occurring amino acid, i.e., an a carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, e.g., homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs have modified R groups (e.g., norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. Amino acid mimetics refers to chemical compounds that have a structure that is different from the general chemical structure of an amino acid, but that functions in a manner similar to a naturally occurring amino acid.

Detailed Description Text (46):
Amino acids may be referred to herein by either their commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Nucleotides, likewise, may be referred to by their commonly accepted single-letter codes.

Detailed Description Text (47):
"Conservatively modified variants" applies to both amino acid and nucleic acid sequences. With respect to particular nucleic acid sequences, conservatively modified variants refers to those nucleic acids which encode identical or essentially identical amino acid sequences, or where the nucleic acid does not encode an amino acid sequence, to essentially identical sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given protein. For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are "silent variations," which are one species of conservatively modified variations. Every nucleic acid sequence herein which encodes a polypeptide also describes every possible silent variation of the nucleic acid. One of skill will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine, and TGG, which is ordinarily the only codon for tryptophan) can be modified to yield a functionally identical molecule.

Accordingly, each silent variation of a nucleic acid which encodes a polypeptide is implicit in each described sequence.

Detailed Description Text (48):
As to amino acid sequences, one of skill will recognize that individual substitutions, deletions or additions to a nucleic acid, peptide, polypeptide, or protein sequence which alters, adds or deletes a single amino acid or a small percentage of amino acids in the encoded sequence is a "conservatively modified variant" where the alteration results in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art. Such conservatively modified variants are in addition to and do not exclude polymorphic variants, interspecies homologs, and alleles of the invention.

Detailed Description Text (49):
The following eight groups each contain amino acids that are conservative substitutions for one another: 1) Alanine (A), Glycine (G); 2) Aspartic acid (D), Glutamic acid (E); 5 3) Asparagine (N), Glutamine (Q); 4) Arginine (R), Lysine (K); 5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W); 7) Serine (S), Threonine (T); and 8) Cysteine (C), Methionine (M) (see, e.g., Creighton, Proteins (1984)).

Detailed Description Text (52):
Any suitable method known in the art can be used to design and construct nucleic acids encoding ZFPs, e.g., phage display, random mutagenesis, combinatorial libraries, computer/rational design, affinity selection, PCR, cloning from cDNA or genomic libraries, synthetic construction and the like. (see, e.g., U.S. Pat. No. 5,786,538; Wu et al., PNAS 92:344-348 (1995); Jamieson et al., Biochemistry 33:5689-5695 (1994); Rebar & Pabo, Science 263:671-673 (1994); Choo & Klug, PNAS 91:11163-11167 (1994); Choo & Klug, PNAS 91:11168-11172 (1994); Desjarlais & Berg, PNAS 90:2256-2260 (1993); Desjarlais & Berg, PNAS 89:7345-7349 (1992); Pomerantz et al., Science 267:93-96 (1995); Pomerantz et al., PNAS 92:9752-9756 (1995); and Liu et al., PNAS 94:5525-5530 (1997); Griesman & Pabo, Science 275:657-661 (1997); Desjarlais & Berg, PNAS 91:11-99-11103 (1994)).

Detailed Description Text (53):
In a preferred embodiment, copending application U.S. Ser. No. 09/229,007, filed Jan. 12, 1999 provides methods that select a target gene, and identify a target site within the gene containing one to six (or more) D-able sites (see definition below). Using these methods, a ZFP can then be synthesized that binds to the preselected site. These methods of target site selection are premised, in part, on the recognition that the presence of one or more D-able sites in a target segment confers the potential for higher binding affinity in a ZFP selected or designed to bind to that site relative to ZFPs that bind to target segments lacking D-able sites (see below). Experimental evidence supporting this insight is provided in Examples 2-9 of copending application U.S. Ser. No. 09/229,007, filed Jan. 12, 1999.

Detailed Description Text (54):
A D-able site or subsite is a region of a target site that allows an appropriately designed single zinc finger to bind to four bases rather than three of the target site. Such a zinc finger binds to a triplet of bases on one strand of a double-stranded target segment (target strand) and a fourth base on the other strand (see FIG. 2 of copending application U.S. Ser. No. 09/229,007, filed Jan. 12, 1999). Binding of a single zinc finger to a four base target segment imposes constraints both on the sequence of the target strand and on the amino acid sequence of the zinc finger. The target site within the target strand should include the "D-able" site motif 5' NNGK 3' (SEQ ID NO:41), in which N and K are conventional IUPAC-IUB ambiguity codes. A zinc finger for binding to such a site should include an arginine residue at position -1 and an aspartic acid, (or less preferably a

glutarnic acid) at position +2. The arginine residues at position -1 interacts with the G residue in the D-able site. The aspartic acid (or glutamic acid) residue at position +2 of the zinc finger interacts with the opposite strand base complementary to the K base in the D-able site. It is the interaction between aspartic acid (symbol D) and the opposite strand base (fourth base)that confers the name D-able site. As is apparent from the D-able site formula, there are two subtypes of D-able sites: 5' NNGG 3' (SEQ ID NO:42) and 5' NNGT 3' (SEQ ID NO:43). For the former site, the aspartic acid or glutamic acid at position +2 of a zinc finger interacts with a C in the opposite strand to the D-able site. In the latter site, the aspartic acid or glutamic acid at position +2 of a zinc finger interacts with an A in the opposite strand to the D-able site. In general, NNGG (SEQ ID NO:42) is preferred over NNGT (SEQ ID NO:43).

Detailed Description Text (55):
In the design of a ZFP with three fingers, a target site should be selected in which at least one finger of the protein, and optionally, two or all three fingers have the potential to bind a D-able site. Such can be achieved by selecting a target site from within a larger target gene having the formula 5'-NNx aNy bNzc-3', wherein each of the sets (x, a), (y, b) and (z, c) is either (N, N) or (G, K); at least one of (x, a), (y, b) and (z, c) is (G, K); and N and K are IUPAC-IUB ambiguity codes

Detailed Description Text (57):
In the formula 5'-NNx aNy bNzc-3', the triplets of NNx aNy and bNzc represent the triplets of bases on the target strand bound by the three fingers in a ZFP. If only one of x, y and z is a G, and this G is followed by a K, the target site includes a single D-able subsite. For example, if only x is G, and a is K, the site reads 5'-NNG KNy bNzc-3' with the D-able subsite highlighted. If both x and y but not z are G, and a and b are K, then the target site has two overlapping D-able subsites as follows: 5'-NNG KNG KNz c-3' (SEQ ID NO:2), with one such site being represented in bold and the other in italics. If all three of x, y and z are G and a, b, and c are K, then the target segment includes three D-able subsites, as follows 5'NNG KNG KNG K3' (SEQ ID NO:3), the D-able subsites being represented by bold, italics and underline.

Detailed Description Text (59):
In a variation, the methods of the invention identify first and second target segments, each independently conforming to the above formula. The two target segments in such methods are constrained to be adjacent or proximate (i.e., within about 0-5 bases) of each other in the target gene. The strategy underlying selection of proximate target segments is to allow the design of a ZFP formed by linkage of two component ZFPs specific for the first and second target segments respectively. These principles can be extended to select target sites to be bound by ZFPs with any number of component fingers. For example, a suitable target site for a nine finger protein would have three component segments, each conforming to the above formula.

Detailed Description Text (60):
The target sites identified by the above methods can be subject to further evaluation by other criteria or can be used directly for design or selection (if needed) and production of a ZFP specific for such a site. A further criteria for evaluating potential target sites is their proximity to particular regions within a gene. If a ZFP is to be used to repress a cellular gene on its own (i.e., without linking the ZFP to a repressing moiety), then the optimal location appears to be at, or within 50 bp upstream or downstream of the site of transcription initiation, to interfere with the formation of the transcription complex (Kim & Pabo, J. Biol. Chem. 272:29795-296800 (1997)) or compete for an essential enhancer binding protein. If, however, a ZFP is fused to a functional domain such as the KRAB repressor domain or the VP16 activator domain, the location of the binding site is considerably more flexible and can be outside known regulatory regions. For

example, a KRAB domain can repress transcription at a promoter up to at least 3 kbp from where KRAB is bound (Margolin et al., PNAS 91:4509-4513 (1994)). Thus, target sites can be selected that do not necessarily include or overlap segments of demonstrable biological significance with target genes, such as regulatory sequences. Other criteria for further evaluating target segments include the prior availability of ZFPs binding to such segments or related segments, and/or ease of designing new ZFPs to bind a given target segment.

Detailed Description Text (61):
After a target segment has been selected, a ZFP that binds to the segment can be provided by a variety of approaches. The simplest of approaches is to provide a precharacterized ZFP from an existing collection that is already known to bind to the target site. However, in many instances, such ZFPs do not exist. An alternative approach can also be used to design new ZFPs, which uses the information in a database of existing ZFPs and their respective binding affinities. A further approach is to design a ZFP based on substitution rules as discussed above. A still further alternative is to select a ZFP with specificity for a given target by an empirical process such as phage display. In some such methods, each component finger of a ZFP is designed or selected independently of other component fingers. For example, each finger can be obtained from a different preexisting ZFP or each finger can be subject to separate randomization and selection.

Detailed Description Text (62):
Once a ZFP has been selected, designed, or otherwise provided to a given target segment, the ZFP or the DNA encoding it are synthesized. Exemplary methods for synthesizing and expressing DNA encoding zinc finger proteins are described below. The ZFP or a polynucleotide encoding it can then be used for modulation of expression, or analysis of the target gene containing the target site to which the ZFP binds.

Detailed Description Text (64):
ZFP polypeptides and nucleic acids can be made using routine techniques in the field of recombinant genetics. Basic texts disclosing the general methods of use in this invention include Sambrook et al., Molecular Cloning, A Laboratory Manual (2nd ed. 1989); Kriegler, Gene Transfer and Expression: A Laboratory Manual (1990); and Current Protocols in Molecular Biology (Ausubel et al., eds., 1994)). In addition, essentially any nucleic acid can be custom ordered from any of a variety of commercial sources. Similarly, peptides and antibodies can be custom ordered from any of a variety of commercial sources.

Detailed Description Text (65):
Two alternative methods are typically used to create the coding sequences required to express newly designed DNA-binding peptides. One protocol is a PCR-based assembly procedure that utilizes six overlapping oligonucleotides (FIG. 1). Three oligonucleotides (oligos 1, 3, and 5 in FIG. 1) correspond to "universal" sequences that encode portions of the DNA-binding domain between the recognition helices. These oligonucleotides remain constant for all zinc finger constructs. The other three "specific" oligonucleotides (oligos 2, 4, and 6 in FIG. 1) are designed to encode the recognition helices. These oligonucleotides contain substitutions primarily at positions -1, 2, 3 and 6 on the recognition helices making them specific for each of the different DNA-binding domains.

Detailed Description Text (66):
The PCR synthesis is carried out in two steps. First, a double stranded DNA template is created by combining the six oligonucleotides (three universal, three specific) in a four cycle PCR reaction with a low temperature annealing step, thereby annealing the oligonucleotides to form a DNA "scaffold." The gaps in the scaffold are filled in by high-fidelity thermostable polymerase, the combination of Taq and Pfu polymerases also suffices. In the second phase of construction, the zinc finger template is amplified by external primers designed to incorporate

restriction sites at either end for cloning into a shuttle vector or directly into an expression vector.

Detailed Description Text (67):
An alternative method of cloning the newly designed DNA-binding proteins relies on annealing complementary oligonucleotides encoding the specific regions of the desired ZFP. This particular application requires that the oligonucleotides be phosphorylated prior to the final ligation step. This is usually performed before setting up the annealing reactions, but kinasing can also occur post-annealing. In brief, the "universal" oligonucleotides encoding the constant regions of the proteins (oligos 1, 2 and 3 of above) are annealed with their complementary oligonucleotides. Additionally, the "specific" oligonucleotides encoding the finger recognition helices are annealed with their respective complementary oligonucleotides. These complementary oligos are designed to fill in the region which was previously filled in by polymerase in the protocol described above. The complementary oligos to the common oligos 1 and finger 3 are engineered to leave overhanging sequences specific for the restriction sites used in cloning into the vector of choice. The second assembly protocol differs from the initial protocol in the following aspects: the "scaffold" encoding the newly designed ZFP is composed entirely of synthetic DNA thereby eliminating the polymerase fill-in step, additionally the fragment to be cloned into the vector does not require amplification. Lastly, the design of leaving sequence-specific overhangs eliminates the need for restriction enzyme digests of the inserting fragment.

Detailed Description Text (69):
Any suitable method of protein purification known to those of skill in the art can be used to purify ZFPs of the invention (see Ausubel, supra, Sambrook, supra). In addition, any suitable host can be used, e.g., bacterial cells, insect cells, yeast cells, mammalian cells, and the like.

Detailed Description Text (70):
In one embodiment, expression of the ZFP fused to a maltose binding protein (MBP-ZFP) in bacterial strain JM109 allows for straightforward purification through an amylose column (NEB). High expression levels of the zinc finger chimeric protein can be obtained by induction with IPTG since the MBP-ZFP fusion in the pMal-c2 expression plasmid is under the control of the IPTG inducible tac promoter (NEB). Bacteria containing the MBP-ZFP fusion plasmids are inoculated in to 2xYT medium containing 10 .mu.M ZnCl.sub.2, 0.02% glucose, plus 50 .mu.g/ml ampicillin and shaken at 37.degree. C. At mid-exponential growth IPTG is added to 0.3 mM and the cultures are allowed to shake. After 3 hours the bacteria are harvested by centrifugation, disrupted by sonication, and then insoluble material is removed by centrifugation. The MBP-ZFP proteins are captured on an amylose-bound resin, washed extensively with buffer containing 20 mM Tris-HCl (pH 7.5), 200 mM NaCl, 5 mM DTT and 50 .mu.M ZnCl.sub.2, then eluted with maltose in essentially the same buffer (purification is based on a standard protocol from NEB). Purified proteins are quantitated and stored for biochemical analysis.

Detailed Description Text (71):
The biochemical properties of the purified proteins, e.g., K.sub.d, can be characterized by any suitable assay. In one embodiment, K.sub.d is characterized via electrophoretic mobility shift assays ("EMSA") (Buratowski & Chodosh, in Current Protocols in Molecular Biology pp. 12.2.1-12.2.7 (Ausubel ed., 1996); see also U.S. Pat. No. 5,789,538, U.S. Ser. No. 09/229,007, filed Jan. 12, 1999, herein incorporated by reference, and Example I). Affinity is measured by titrating purified protein against a low fixed amount of labeled double-stranded oligonucleotide target. The target comprises the natural binding site sequence (9 or 18 bp) flanked by the 3 bp found in the natural sequence. External to the binding site plus flanking sequence is a constant sequence. The annealed oligonucleotide targets possess a 1 bp 5' overhang which allows for efficient labeling of the target with T4 phage polynucleotide kinase. For the assay the

target is added at a concentration of 40 nM or lower (the actual concentration is kept at least 10-fold lower than the lowest protein dilution) and the reaction is allowed to equilibrate for at least 45 min. In addition the reaction mixture also contains 10 mM Tris (pH 7.5), 100 mM KCl, 1 mM MgCl.sub.2, 0.1 mM ZnCl.sub.2, 5 mM DTT, 10% glycerol, 0.02% BSA (poly (dIdC) or (dAdT) (Pharmacia) can also added at 10-100 .mu.g/.mu.l).

Detailed Description Text (72):
The equilibrated reactions are loaded onto a 10% polyacrylamide gel, which has been pre-run for 45 min in Tris/glycine buffer, then bound and unbound labeled target is resolved be electrophoresis at 150V (alternatively, 10-20% gradient Tris-HCl gels, containing a 4% polyacrylamide stacker, can be used). The dried gels are visualized by autoradiography or phosphoroimaging and the apparent K.sub.d is determined by calculating the protein concentration that gives half-maximal binding.

Detailed Description Text (73):
Similar assays can also include determining active fractions in the protein preparations. Active fractions are determined by stoichiometric gel shifts where proteins are titrated against a high concentration of target DNA. Titrations are done at 100, 50, and 25% of target (usually at micromolar levels).

Detailed Description Text (74):
In another embodiment, phage display libraries can be used to select ZFPs with high affinity to the selected target site. This method differs fundamentally from direct design in that it involves the generation of diverse libraries of mutagenized ZFPs, followed by the isolation of proteins with desired DNA-binding properties using affinity selection methods. To use this method, the experimenter typically proceeds as follows.

Detailed Description Text (75):
First, a gene for a ZFP is mutagenized to introduce diversity into regions important for binding specificity and/or affinity. In a typical application, this is accomplished via randomization of a single finger at positions -1, +2, +3, and +6, and perhaps accessory positions such as +1, +5, +8, or +10.

Detailed Description Text (76):
Next, the mutagenized gene is cloned into a phage or phagemid vector as a fusion with, e.g., gene III of filamentous phage, which encodes the coat protein pIII. The zinc finger gene is inserted between segments of gene III encoding the membrane export signal peptide and the remainder of pIII, so that the ZFP is expressed as an amino-terminal fusion with pIII in the mature, processed protein. When using phagemid vectors, the mutagenized zinc finger gene may also be fused to a truncated version of gene III encoding, minimally, the C-terminal region required for assembly of pIII into the phage particle.

Detailed Description Text (77):
The resultant vector library is transformed into E. coli and used to produce filamentous phage which express variant ZFPs on their surface as fusions with the coat protein pIII (if a phagemid vector is used, then the this step requires superinfection with helper phage). The phage library is then incubated with target DNA site, and affinity selection methods are used to isolate phage which bind target with high affinity from bulk phage. Typically, the DNA target is immobilized on a solid support, which is then washed under conditions sufficient to remove all but the tightest binding phage. After washing, any phage remaining on the support are recovered via elution under conditions which totally disrupt zinc finger-DNA binding.

Detailed Description Text (78):
Recovered phage are used to infect fresh E. coli, which is then amplified and used to produce a new batch of phage particles. The binding and recovery steps are then

repeated as many times as is necessary to sufficiently enrich the phage pool for tight binders such that these may be identified using sequencing and/or screening methods.

Detailed Description Text (80):
The ZFPs of the invention can optionally be associated with regulatory domains for modulation of gene expression. The ZFP can be covalently or non-covalently associated with one or more regulatory domains, alternatively two or more regulatory domains, with the two or more domains being two copies of the same domain, or two different domains. The regulatory domains can be covalently linked to the ZFP, e.g., via an amino acid linker, as part of a fusion protein. The ZFPs can also be associated with a regulatory domain via a non-covalent dimerization domain, e.g., a leucine zipper, a STAT protein N terminal domain, or an FK506 binding protein (see, e.g., O'Shea, Science 254:539 (1991), Barahmand-Pour et al., Curr. Top. Microbiol. Immunol. 211:121-128 (1996); Klemm et al., Annu. Rev. Immunol. 16:569-592 (1998); Klemm et al., Annu. Rev. Immunol. 16:569-592 (1998); Ho et al., Nature 382:822-826 (1996); and Pomeranz et al., Biochem. 37:965 (1998)). The regulatory domain can be associated with the ZFP at any suitable position, including the C- or N-terminus of the ZFP.

Detailed Description Text (81):
Common regulatory domains for addition to the ZFP include, e.g., effector domains from transcription factors (activators, repressors, co-activators, co-repressors), silencers, nuclear hormone receptors, oncogene transcription factors (e.g., myc, jun, fos, myb, max, mad, rel, ets, bcl, mos family members etc.); DNA repair enzymes and their associated factors and modifiers; DNA rearrangement enzymes and their associated factors and modifiers; chromatin associated proteins and their modifiers (e.g., kinases, acetylases and deacetylases); and DNA modifying enzymes (e.g., methyltransferases, topoisomerases, helicases, ligases, kinases, phosphatases, polymerases, endonucleases) and their associated factors and modifiers.

Detailed Description Text (82):
Transcription factor polypeptides from which one can obtain a regulatory domain include those that are involved in regulated and basal transcription. Such polypeptides include transcription factors, their effector domains, coactivators, silencers, nuclear hormone receptors (see, e.g., Goodrich et al., Cell 84:825-30 (1996) for a review of proteins and nucleic acid elements involved in transcription; transcription factors in general are reviewed in Barnes & Adcock, Clin. Exp. Allergy 25 Suppl. 2:46-9 (1995) and Roeder, Methods Enzymol. 273:165-71 (1996)). Databases dedicated to transcription factors are known (see, e.g., Science 269:630 (1995)). Nuclear hormone receptor transcription factors are described in, for example, Rosen et al., J. Med. Chem. 38:4855-74 (1995). The C/EBP family of transcription factors are reviewed in Wedel et al., Immunobiology 193:171-85 (1995). Coactivators and co-repressors that mediate transcription regulation by nuclear hormone receptors are reviewed in, for example, Meier, Eur. J. Endocrinol. 134(2):158-9 (1996); Kaiser et al., Trends Biochem. Sci. 21:342-5 (1996); and Utley et al., Nature 394:498-502 (1998)). GATA transcription factors, which are involved in regulation of hematopoiesis, are described in, for example, Simon, Nat. Genet. 11:9-11 (1995); Weiss et al., Exp. Hematol. 23:99-107. TATA box binding protein (TBP) and its associated TAF polypeptides (which include TAF30, TAF55, TAF80, TAF110, TAF150, and TAF250) are described in Goodrich & Tjian, Curr. Opin. Cell Biol. 6:403-9 (1994) and Hurley, Curr. Opin. Struct. Biol. 6:69-75 (1996). The STAT family of transcription factors are reviewed in, for example, Barahmand-Pour et al., Curr. Top. Microbiol. Immunol. 211:121-8 (1996). Transcription factors involved in disease are reviewed in Aso et al., J. Clin. Invest. 97:1561-9 (1996).

Detailed Description Text (83):
In one embodiment, the KRAB repression domain from the human KOX-1 protein is used as a transcriptional repressor (Thiesen et al., New Biologist 2:363-374 (1990);

Margolin et al., PNAS 91:4509-4513 (1994); Pengue et al., Nucl. Acids Res. 22:2908-2914 (1994); Witzgall et al., PNAS 91:4514-4518 (1994); see also Example III)). In another embodiment, KAP-1, a KRAB co-repressor, is used with KRAB (Friedman et al., Genes Dev. 10:2067-2078 (1996)). Alternatively, KAP-1 can be used alone with a ZFP. Other preferred transcription factors and transcription factor domains that act as transcriptional repressors include MAD (see, e.g., Sommer et al., J. Biol. Chem. 273:6632-6642 (1998); Gupta et al., Oncogene 16:1149-1159 (1998); Queva et al., Oncogene 16:967-977 (1998); Larsson et al., Oncogene 15:737-748 (1997); Laherty et al., Cell 89:349-356 (1997); and Cultraro et al., Mol Cell. Biol. 17:2353-2359 (19977)); FKHR (forkhead in rhapdosarcoma gene; Ginsberg et al., Cancer Res. 15:3542-3546 (1998); Epstein et al., Mol. Cell. Biol. 18:4118-4130 (1998)); EGR-1 (early growth response gene product-1; Yan et al., PNAS 95:8298-8303 (1998); and Liu et al., Cancer Gene Ther. 5:3-28 (1998)); the ets2 repressor factor repressor domain (ERD; Sgouras et al., EMBO J. 14:4781-4793 ((19095)); and the MAD smSIN3 interaction domain (SID; Ayer et al., Mol. Cell. Biol. 16:5772-5781 (1996)).

Detailed Description Text (85):
Kinases, phosphatases, and other proteins that modify polypeptides involved in gene regulation are also useful as regulatory domains for ZFPs. Such modifiers are often involved in switching on or off transcription mediated by, for example, hormones. Kinases involved in transcription regulation are reviewed in Davis, Mol. Reprod. Dev. 42:459-67 (1995), Jackson et al., Adv. Second Messenger Phosphoprotein Res. 28:279-86 (1993), and Boulikas, Crit. Rev. Eukaryot. Gene Expr. 5:1-77 (1995), while phosphatases are reviewed in, for example, Schonthal & Semin, Cancer Biol. 6:239-48 (1995). Nuclear tyrosine kinases are described in Wang, Trends Biochem. Sci. 19:373-6 (1994).

Detailed Description Text (88):
Similarly, regulatory domains can be derived from DNA modifying enzymes (e.g., DNA methyltransferases, topoisomerases, helicases, ligases, kinases, phosphatases, polymerases) and their associated factors and modifiers. Helicases are reviewed in Matson et al., Bioessays, 16:13-22 (1994), and methyltransferases are described in Cheng, Curr. Opin. Struct. Biol. 5:4-10 (1995). Chromatin associated proteins and their modifiers (e.g., kinases, acetylases and deacetylases), such as histone deacetylase (Wolffe, Science 272:371-2 (1996)) are also useful as domains for-addition to the ZFP of choice. In one preferred embodiment, the regulatory domain is a DNA methyl transferase that acts as a transcriptional repressor (see, e.g., Van den Wyngaert et al., FEBS Lett. 426:283-289 (1998); Flynn et al., J. Mol. Biol. 279:101-116 (1998); Okano et al., Nucleic Acids Res. 26:2536-2540 (1998); and Zardo & Caiafa, J. Biol. Chem. 273:16517-16520 (1998)). In another preferred embodiment, endonucleases such as Fok1 are used as transcriptional repressors, which act via gene cleavage (see, e.g., WO95/09233; and PCT/US94/01201).

Detailed Description Text (89):
Factors that control chromatin and DNA structure, movement and localization and their associated factors and modifiers; factors derived from microbes (e.g., prokaryotes, eukaryotes and virus) and factors that associate with or modify them can also be used to obtain chimeric proteins. In one embodiment, recombinases and integrases are used as regulatory domains. In one embodiment, histone acetyltransferase is used as a transcriptional activator (see, e.g., Jin & Scotto, Mol. Cell. Biol. 18:4377-4384 (1998); Wolffe, Science 272:371-372 (1996); Taunton et al., Science 272:408-411 (1996); and Hassig et al., PNAS 95:3519-3524 (1998)). In another embodiment, histone deacetylase is used as a transcriptional repressor (see, e.g., Jin & Scotto, Mol. Cell. Biol. 18:4377-4384 (1998); Syntichaki & Thireos, J. Biol. Chem. 273:24414-24419 (1998); Sakaguchi et al., Genes Dev. 12:2831-2841 (1998); and Martinez et al., J. Biol. Chem. 273:23781-23785 (1998)).

Detailed Description Text (90):
Linker domains between polypeptide domains, e.g., between two ZFPs or between a ZFP and a regulatory domain, can be included. Such linkers are typically polypeptide

sequences, such as polyglycine sequences of between about 5 and 200 amino acids. Preferred linkers are typically flexible amino acid subsequences which are synthesized as part of a recombinant fusion protein. For example, in one embodiment, the linker DGGGS (SEQ ID NO:4) is used to link two ZFPs. In another embodiment, the flexible linker linking two ZFPs is an amino acid subsequence comprising the sequence TGEKP (SEQ ID NO:5) (see, e.g., Liu et al., PNAS 5525-5530 (1997)). In another embodiment, the linker LRQKDGERP (SEQ ID NO:6) is used to link two ZFPs. In another embodiment, the following linkers are used to link two ZFPs: GGRR (SEQ ID NO:7) (Pomerantz et al. 1995, supra), (G4S).sub.n (SEQ ID NO:8) (Kim et al., PNAS 93, 1156-1160 (1996.); and GGRRGGGS (SEQ ID NO:9); LRQRDGERP (SEQ ID NO:10); LRQKDGGGSERP (SEQ ID NO:11); LRQKd(G3S).sub.2 ERP (SEQ ID NO:12). Alternatively, flexible linkers can be rationally designed using computer program capable of modeling both DNA-binding sites and the peptides themselves (Desjarlais & Berg, PNAS 90:2256-2260 (1993), PNAS 91:11099-11103 (1994) or by phage display methods.

Detailed Description Text (92):
In addition to regulatory domains, often the ZFP is expressed as a fusion protein such as maltose binding protein ("MBP"), glutathione S transferase (GST), hexahistidine, c-myc, and the cellular and subcellular localization.

Detailed Description Text (93):
Expression Vectors for Nucleic Acids Encoding ZFP

Detailed Description Text (94):
The nucleic acid encoding the ZFP of choice is typically cloned into intermediate vectors for transformation into prokaryotic or eukaryotic cells for replication and/or expression, e.g., for determination of K.sub.d. Intermediate vectors are typically prokaryote vectors, e.g., plasmids, or shuttle vectors, or insect vectors, for storage or manipulation of the nucleic acid encoding ZFP or production of protein. The nucleic acid encoding a ZFP is also typically cloned into an expression vector, for administration to a plant cell, animal cell, preferably a mammalian cell or a human cell, fungal cell, bacterial cell, or protozoal cell.

Detailed Description Text (95):
To obtain expression of a cloned gene or nucleic acid, a ZFP is typically subcloned into an expression vector that contains a promoter to direct transcription. Suitable bacterial and eukaryotic promoters are well known in the art and described, e.g., in Sambrook et al., Molecular Cloning, A Laboratory Manual (2nd ed. 1989); Kriegler, Gene Transfer and Expression: A Laboratory Manual (1990); and Current Protocols in Molecular Biology (Ausubel et al., eds., 1994). Bacterial expression systems for expressing the ZFP are available in, e.g., E. coli, Bacillus sp., and Salmonella (Palva et al., Gene 22:229-235 (1983)). Kits for such expression systems are commercially available. Eukaryotic expression systems for mammalian cells, yeast, and insect cells are well known in the art and are also commercially available.

Detailed Description Text (96):
The promoter used to direct expression of a ZFP nucleic acid depends on the particular application. For example, a strong constitutive promoter is typically used for expression and purification of ZFP. In contrast, when a ZFP is administered in vivo for gene regulation, either a constitutive or an inducible promoter is used, depending on the particular use of the ZFP. In addition, a preferred promoter for administration of a ZFP can be a weak promoter, such as HSV TK or a promoter having similar activity. The promoter typically can also include elements that are responsive to transactivation, e.g., hypoxia response elements, Gal4 response elements, lac repressor response element, and small molecule control systems such as tet-regulated systems and the RU-486 system (see, e.g., Gossen & Bujard, PNAS 89:5547 (1992); Oligino et al., Gene Ther. 5:491-496 (1998); Wang et al., Gene Ther. 4:432-441 (1997); Neering et al., Blood 88:1147-1155 (1996); and

Rendahl et al., Nat. Biotechnol. 16:757-761 (1998)).

Detailed Description Text (97):
In addition to the promoter, the expression vector typically contains a transcription unit or expression cassette that contains all the additional elements required for the expression of the nucleic acid in host cells, either prokaryotic or eukaryotic. A typical expression cassette thus contains a promoter operably linked, e.g., to the nucleic acid sequence encoding the ZFP, and signals required, e.g., for efficient polyadenylation of the transcript, transcriptional termination, ribosome binding sites, or translation termination. Additional elements of the cassette may include, e.g., enhancers, and heterologous spliced intronic signals.

Detailed Description Text (98):
The particular expression vector used to transport the genetic information into the cell is selected with regard to the intended use of the ZFP, e.g., expression in plants, animals, bacteria, fungus, protozoa etc. (see expression vectors described below and in the Example section). Standard bacterial expression vectors include plasmids such as pBR322 based plasmids, pSKF, pET23D, and commercially available fusion expression systems such as GST and LacZ. A preferred fusion protein is the maltose binding protein, "MBP." Such fusion proteins are used for purification of the ZFP. Epitope tags can also be added to recombinant proteins to provide convenient methods of isolation, for monitoring expression, and for monitoring cellular and subcellular localization, e.g., c-myc or FLAG.

Detailed Description Text (99):
Expression vectors containing regulatory elements from eukaryotic viruses are often used in eukaryotic expression vectors, e.g., SV40 vectors, papilloma virus vectors, and vectors derived from Epstein-Barr virus. Other exemplary eukaryotic vectors include pMSG, pAV009/A+, pMTO10/A+, pMAMneo-5, baculovirus pDSVE, and any other vector allowing expression of proteins under the direction of the SV40 early promoter, SV40 late promoter, metallothionein promoter, murine mammary tumor virus promoter, Rous sarcoma virus promoter, polyhedrin promoter, or other promoters shown effective for expression in eukaryotic cells.

Detailed Description Text (102):
Standard transfection methods are used to produce bacterial, mammalian, yeast or insect cell lines that express large quantities of protein, which are then purified using standard techniques (see, e.g., Colley et al., J. Biol. Chem. 264:17619-17622 (1989); Guide to Protein Purification, in Methods in Enzymology, vol. 182 (Deutscher, ed., 1990)). Transformation of eukaryotic and prokaryotic cells are performed according to standard techniques (see, e.g., Morrison, J. Bact. 132:349-351 (1977); Clark-Curtiss & Curtiss, Methods in Enzymology 101:347-362 (Wu et al., eds, 1983).

Detailed Description Text (103):
Any of the well known procedures for introducing foreign nucleotide sequences into host cells may be used. These include the use of calcium phosphate transfection, polybrene, protoplast fusion, electroporation, liposomes, microinjection, naked DNA, plasmid vectors, viral vectors, both episomal and integrative, and any of the other well known methods for introducing cloned genomic DNA, cDNA, synthetic DNA or other foreign genetic material into a host cell (see, e.g., Sambrook et al., supra). It is only necessary that the particular genetic engineering procedure used be capable of successfully introducing at least one gene into the host cell capable of expressing the protein of choice.

Detailed Description Text (105):
A variety of assays can be used to determine the level of gene expression regulation by ZFPs. The activity of a particular ZFP can be assessed using a variety of in vitro and in vivo assays, by measuring, e.g., protein or mRNA levels, product levels, enzyme activity, tumor growth; transcriptional activation or

repression of a reporter gene; second messenger levels (e.g., cGMP, cAMP, IP3, DAG, Ca.sup.2+); cytokine and hormone production levels; and neovascularization, using, e.g., immunoassays (e.g., ELISA and immunohistochemical assays with antibodies), hybridization assays (e.g., RNase protection, northerns, in situ hybridization, oligonucleotide array studies), colorimetric assays, amplification assays, enzyme activity assays, tumor growth assays, phenotypic assays, and the like.

Detailed Description Text (106):
ZFPs are typically first tested for activity in vitro using cultured cells, e.g., 293 cells, CHO cells, VERO cells, BHK cells, HeLa cells, COS cells, and the like. Preferably, human cells are used. The ZFP is often first tested using a transient expression system with a reporter gene, and then regulation of the target endogenous gene is tested in cells and in animals, both in vivo and ex vivo. The ZFP can be recombinantly expressed in a cell, recombinantly expressed in cells transplanted into an animal, or recombinantly expressed in a transgenic animal, as well as administered as a protein to an animal or cell using delivery vehicles described below. The cells can be immobilized, be in solution, be injected into an animal, or be naturally occurring in a transgenic or non-transgenic animal.

Detailed Description Text (109):
Preferred assays for ZFP regulation of endogenous gene expression can be performed in vitro. In one preferred in vitro assay format, ZFP regulation of endogenous gene expression in cultured cells is measured by examining protein production using an ELISA assay (see Examples VI and VII). The test sample is compared to control cells treated with an empty vector or an unrelated ZFP that is targeted to another gene.

Detailed Description Text (110):
In another embodiment, ZFP regulation of endogenous gene expression is determined in vitro by measuring the level of target gene mRNA expression. The level of gene expression is measured using amplification, e.g., using PCR, LCR, or hybridization assays, e.g., northern hybridization, RNase protection, dot blotting. RNase protection is used in one embodiment (see Example VIII and FIG. 10). The level of protein or mRNA is detected using directly or indirectly labeled detection agents, e.g., fluorescently or radioactively labeled nucleic acids, radioactively or enzymatically labeled antibodies, and the like, as described herein.

Detailed Description Text (111):
Alternatively, a reporter gene system can be devised using the target gene promoter operably linked to a reporter gene such as luciferase, green fluorescent protein, CAT, or .beta.-gal. The reporter construct is typically co-transfected into a cultured cell. After treatment with the ZFP of choice, the amount of reporter gene transcription, translation, or activity is measured according to standard techniques known to those of skill in the art.

Detailed Description Text (114):
Nucleic Acids Encoding ZFPs and Gene Therapy

Detailed Description Text (115):
Conventional viral and non-viral based gene transfer methods can be used to introduce nucleic acids encoding engineered ZFP in mammalian cells or target tissues. Such methods can be used to administer nucleic acids encoding ZFPs to cells in vitro. Preferably, the nucleic acids encoding ZFPs are administered for in vivo or ex vivo gene therapy uses. Non-viral vector delivery systems include DNA plasmids, naked nucleic acid, and nucleic acid complexed with a delivery vehicle such as a liposome. Viral vector delivery systems include DNA and RNA viruses, which have either episomal or integrated genomes after delivery to the cell. For a review of gene therapy procedures, see Anderson, Science 256:808-813 (1992); Nabel & Felgner, TIBTECH 11:211-217 (1993); Mitani & Caskey, TIBTECH 11:162-166 (1993); Dillon, TIBTECH 11:167-175 (1993); Miller, Nature 357:455-460 (1992); Van Brunt, Biotechnology 6(10):1149-1154 (1988); Vigne, Restorative Neurology and Neuroscience

8:35-36 (1995); Kremer & Perricaudet, British Medical Bulletin 51(1):31-44 (1995); Haddada et al., in Current Topics in Microbiology and Immunology Doerfler and Bohm (eds) (1995); and Yu et al., Gene Therapy 1:13-26 (1994).

Detailed Description Text (116):
Methods of non-viral delivery of nucleic acids encoding engineered ZFPs include lipofection, microinjection, ballistics, virosomes, liposomes, immunoliposomes, polycation or lipid:nucleic acid conjugates, naked DNA, artificial virions, and agent-enhanced uptake of DNA. Lipofection is described in e.g., U.S. Pat. No. 5,049,386, U.S. Pat. No. 4,946,787; and U.S. Pat. No. 4,897,355) and lipofection reagents are sold commercially (e.g., Transfectam.TM. and Lipofectin.TM.). Cationic and neutral lipids that are suitable for efficient receptor-recognition lipofection of polynucleotides include those of Feigner, WO 91/17424, WO 91/16024. Delivery can be to cells (ex vivo administration) or target tissues (in vivo administration).

Detailed Description Text (117):
The preparation of lipid:nucleic acid complexes, including targeted liposomes such as immunolipid complexes, is well known to one of skill in the art (see, e.g., Crystal, Science 270:404-410 (1995); Blaese et al., Cancer Gene Ther. 2:291-297 (1995); Behr et al., Bioconjugate Chem. 5:382-389 (1994); Remy et al., Bioconjugate Chem. 5:647-654 (1994); Gao et al., Gene Therapy 2:710-722 (1995); Ahmad et al., Cancer Res. 52:4817-4820 (1992); U.S. Pat. Nos. 4,186,183, 4,217,344, 4,235,871, 4,261,975, 4,485,054, 4,501,728, 4,774,085, 4,837,028, and 4,946,787).

Detailed Description Text (118):
The use of RNA or DNA viral based systems for the delivery of nucleic acids encoding engineered ZFP take advantage of highly evolved processes for targeting a virus to specific cells in the body and trafficking the viral payload to the nucleus. Viral vectors can be administered directly to patients (in vivo) or they can be used to treat cells in vitro and the modified cells are administered to patients (ex vivo). Conventional viral based systems for the delivery of ZFPs could include retroviral, lentivirus, adenoviral, adeno-associated and herpes simplex virus vectors for gene transfer. Viral vectors are currently the most efficient and versatile method of gene transfer in target cells and tissues. Integration in the host genome is possible with the retrovirus, lentivirus, and adeno-associated virus gene transfer methods, often resulting in long term expression of the inserted transgene. Additionally, high transduction efficiencies have been observed in many different cell types and target tissues.

Detailed Description Text (119):
The tropism of a retrovirus can be altered by incorporating foreign envelope proteins, expanding the potential target population of target cells. Lentiviral vectors are retroviral vector that are able to transduce or infect non-dividing cells and typically produce high viral titers. Selection of a retroviral gene transfer system would therefore depend on the target tissue. Retroviral vectors are comprised of cis-acting long terminal repeats with packaging capacity for up to 6-10 kb of foreign sequence. The minimum cis-acting LTRs are sufficient for replication and packaging of the vectors, which are then used to integrate the therapeutic gene into the target cell to provide permanent transgene expression. Widely used retroviral vectors include those based upon murine leukemia virus (MuLV), gibbon ape leukemia virus (GaLV), Simian Immuno deficiency virus (SIV), human immuno deficiency virus (HIV), and combinations thereof (see, e.g., Buchscher et al., J. Virol. 66:2731-2739 (1992); Johann et al., J. Virol. 66:1635-1640 (1992); Sommerfelt et al., Virol. 176:58-59 (1990); Wilson et al., J. Virol. 63:2374-2378 (1989); Miller et al., J. Virol. 65:2220-2224 (1991); PCT/US94/05700).

Detailed Description Text (120):
In applications where transient expression of the ZFP is preferred, adenoviral based systems are typically used. Adenoviral based vectors are capable of very high

transduction efficiency in many cell types and do not require cell division. With such vectors, high titer and levels of expression have been obtained. This vector can be produced in large quantities in a relatively simple system. Adeno-associated virus ("AAV") vectors are also used to transduce cells with target nucleic acids, e.g., in the in vitro production of nucleic acids and peptides, and for in vivo and ex vivo gene therapy procedures (see, e.g., West et al., Virology 160:38-47 (1987); U.S. Pat. No. 4,797,368; WO 93/24641; Kotin, Human Gene Therapy 5:793-801 (1994); Muzyczka, J. Clin. Invest. 94:1351 (1994). Construction of recombinant AAV vectors are described in a number of publications, including U.S. Pat. No. 5,173,414; Tratschin et al., Mol. Cell. Biol. 5:3251-3260 (1985); Tratschin, et al., Mol. Cell. Biol. 4:2072-2081 (1984); Hermonat & Muzyczka, PNAS 81:6466-6470 (1984); and Samulski et al., J. Virol. 63:03822-3828 (1989).

Detailed Description Text (125):
Packaging cells are used to form virus particles that are capable of infecting a host cell. Such cells include 293 cells, which package adenovirus, and .psi.2 cells or PA317 cells, which package retrovirus. Viral vectors used in gene therapy are usually generated by producer cell line that packages a nucleic acid vector into a viral particle. The vectors typically contain the minimal viral sequences required for packaging and subsequent integration into a host, other viral sequences being replaced by an expression cassette for the protein to be expressed. The missing viral functions are supplied in trans by the packaging cell line. For example, AAV vectors used in gene therapy typically only possess ITR sequences from the AAV genome which are required for packaging and integration into the host genome. Viral DNA is packaged in a cell line, which contains a helper plasmid encoding the other AAV genes, namely rep and cap, but lacking ITR sequences. The cell line is also infected with adenovirus as a helper. The helper virus promotes replication of the AAV vector and expression of AAV genes from the helper plasmid. The helper plasmid is not packaged in significant amounts due to a lack of ITR sequences. Contamination with adenovirus can be reduced by, e.g., heat treatment to which adenovirus is more sensitive than AAV.

Detailed Description Text (126):
In many gene therapy applications, it is desirable that the gene therapy vector be delivered with a high degree of specificity to a particular tissue type. A viral vector is typically modified to have specificity for a given cell type by expressing a ligand as a fusion protein with a viral coat protein on the viruses outer surface. The ligand is chosen to have affinity for a receptor known to be present on the cell type of interest. For example, Han et al., PNAS 92:9747-9751 (1995), reported that Moloney murine leukemia virus can be modified to express human heregulin fused to gp70, and the recombinant virus infects certain human breast cancer cells expressing human epidermal growth factor receptor. This principle can be extended to other pairs of virus expressing a ligand fusion protein and target cell expressing a receptor. For example, filamentous phage can be engineered to display antibody fragments (e.g., FAB or Fv) having specific binding affinity for virtually any chosen cellular receptor. Although the above description applies primarily to viral vectors, the same principles can be applied to nonviral vectors. Such vectors can be engineered to contain specific uptake sequences thought to favor uptake by specific target cells.

Detailed Description Text (128):
Ex vivo cell transfection for diagnostics, research, or for gene therapy (e.g., via re-infusion of the transfected cells into the host organism) is well known to those of skill in the art. In a preferred embodiment, cells are isolated from the subject organism, transfected with a ZFP nucleic acid (gene or cDNA), and re-infused back into the subject organism (e.g., patient). Various cell types suitable for ex vivo transfection are well known to those of skill in the art (see, e.g., Freshney et al., Culture of Animal Cells, A Manual of Basic Technique (3rd ed. 1994)) and the references cited therein for a discussion of how to isolate and culture cells from patients).

Detailed Description Text (131):
Vectors (e.g., retroviruses, adenoviruses, liposomes, etc.) containing therapeutic ZFP nucleic acids can be also administered directly to the organism for transduction of cells in vivo. Alternatively, naked DNA can be administered. Administration is by any of the routes normally used for introducing a molecule into ultimate contact with blood or tissue cells. Suitable methods of administering such nucleic acids are available and well known to those of skill in the art, and, although more than one route can be used to administer a particular composition, a particular route can often provide a more immediate and more effective reaction than another route.

Detailed Description Text (134):
An important factor in the administration of polypeptide compounds, such as the ZFPs, is ensuring that the polypeptide has the ability to traverse the plasma membrane of a cell, or the membrane of an intra-cellular compartment such as the nucleus. Cellular membranes are composed of lipid-protein bilayers that are freely permeable to small, nonionic lipophilic compounds and are inherently impermeable to polar compounds, macromolecules, and therapeutic or diagnostic agents. However, proteins and other compounds such as liposomes have been described, which have the ability to translocate polypeptides such as ZFPs across a cell membrane.

Detailed Description Text (135):
For example, "membrane translocation polypeptides" have amphiphilic or hydrophobic amino acid subsequences that have the ability to act as membrane-translocating carriers. In one embodiment, homeodomain proteins have the ability to translocate across cell membranes. The shortest intemalizable peptide of a homeodomain protein, Antennapedia, was found to be the third helix of the protein, from amino acid position 43 to 58 (see, e.g., Prochiantz, Current Opinion in Neurobiology 6:629-634 (1996)). Another subsequence, the h (hydrophobic) domain of signal peptides, was found to have similar cell membrane translocation characteristics (see, e.g., Lin et al., J. Biol. Chem. 270:1 4255-14258 (1995)).

Detailed Description Text (136):
Examples of peptide sequences which can be linked to a ZFP of the invention, for facilitating uptake of ZFP into cells, include, but are not limited to: an 11 animo acid peptide of the tat protein of HIV; a 20 residue peptide sequence which corresponds to amino acids 84-103 of the p16 protein (see Fahraeus et al., Current Biology 6:84 (1996)); the third helix of the 60-amino acid long homeodomain of Antennapedia (Derossi et al., J. Biol. Chem. 269:10444 (1994)); the h region of a signal peptide such as the Kaposi fibroblast growth factor (K-FGF) h region (Lin et al., supra); or the VP22 translocation domain from HSV (Elliot & O'Hare, Cell 88:223-233 (1997)). Other suitable chemical moieties that provide enhanced cellular uptake may also be chemically linked to ZFPs.

Detailed Description Text (137):
Toxin molecules also have the ability to transport polypeptides across cell membranes. Often, such molecules are composed of at least two parts (called "binary toxins"): a translocation or binding domain or polypeptide and a separate toxin domain or polypeptide. Typically, the translocation domain or polypeptide binds to a cellular receptor, and then the toxin is transported into the cell. Several bacterial toxins, including Clostridium perfringens iota toxin, diphtheria toxin (DT), Pseudomonas exotoxin A (PE), pertussis toxin (PT), Bacillus anthracis toxin, and pertussis adenylate cyclase (CYA), have been used in attempts to deliver peptides to the cell cytosol as internal or amino-terminal fusions (Arora et al., J. Biol. Chem., 268:3334-3341 (1993); Perelle et al., Infect. Immun., 61:5147-5156 (1993); Stenmark et al., J. Cell Biol. 113:1025-1032 (1991); Donnelly et al., PNAS 90:3530-3534 (1993); Carbonetti et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 95:295 (1995); Sebo et al., Infect. Immun. 63:3851-3857 (1995); Klimpel et al., PNAS U.S.A. 89:10277-10281 (1992); and Novak et al., J. Biol. Chem. 267:17186-17193

1992)).

Detailed Description Text (138):
Such subsequences can be used to translocate ZFPs across a cell membrane. ZFPs can be conveniently fused to or derivatized with such sequences. Typically, the translocation sequence is provided as part of a fusion protein. Optionally, a linker can be used to link the ZFP and the translocation sequence. Any suitable linker can be used, e.g., a peptide linker.

Detailed Description Text (145):
Standard methods for coupling targeting agents to liposomes can be used. These methods generally involve incorporation into liposomes lipid components, e.g., phosphatidylethanolamine, which can be activated for attachment of targeting agents, or derivatized lipophilic compounds, such as lipid derivatized bleomycin. Antibody targeted liposomes can be constructed using, for instance, liposomes which incorporate protein A (see Renneisen et al., J. Biol. Chem., 265:16337-16342 (1990) and Leonetti et al., PNAS 87:2448-2451 (1990).

Detailed Description Text (148):
The maximum therapeutically effective dosage of ZFP for approximately 99% binding to target sites is calculated to be in the range of less than about 1.5.times.10.sup.5 to 1.5.times.10.sup.6 copies of the specific ZFP molecule per cell. The number of ZFPs per cell for this level of binding is calculated as follows, using the volume of a HeLa cell nucleus (approximately 1000 .mu.m.sup.3 or 10.sup.-12 L; Cell Biology, (Altman & Katz, eds. (1976)). As the HeLa nucleus is relatively large, this dosage number is recalculated as needed using the volume of the target cell nucleus. This calculation also does not take into account competition for ZFP binding by other sites. This calculation also assumes that essentially all of the ZFP is localized to the nucleus. A value of 100.times.K.sub.d is used to calculate approximately 99% binding of to the target site, and a value of 10.times.K.sub.d is used to calculate approximately 90% binding of to the target site. For this example, K.sub.d =25 nM

Detailed Description Text (151):
In determining the effective amount of the ZFP to be administered in the treatment or prophylaxis of disease, the physician evaluates circulating plasma levels of the ZFP or nucleic acid encoding the ZFP, potential ZFP toxicities, progression of the disease, and the production of anti-ZFP antibodies. Administration can be accomplished via single or divided doses.

Detailed Description Text (159):
ZFPs can be used to engineer plants for traits such as increased disease resistance, modification of structural and storage polysaccharides, flavors, proteins, and fatty acids, fruit ripening, yield, color, nutritional characteristics, improved storage capability, and the like. In particular, the engineering of crop species for enhanced oil production, e.g., the modification of the fatty acids produced in oilseeds, is of interest.

Detailed Description Text (160):
Seed oils are composed primarily of triacylglycerols (TAGs), which are glycerol esters of fatty acids. Commercial production of these vegetable oils is accounted for primarily by six major oil crops (soybean, oil palm, rapeseed, sunflower, cotton seed, and peanut.) Vegetable oils are used predominantly (90%) for human consumption as margarine, shortening, salad oils, and frying oil. The remaining 10% is used for non-food applications such as lubricants, oleochemicals, biofuels, detergents, and other industrial applications.

Detailed Description Text (161):
The desired characteristics of the oil used in each of these applications varies widely, particularly in terms of the chain length and number of double bonds

present in the fatty acids making up the TAGs. These properties are manipulated by the plant in order to control membrane fluidity and temperature sensitivity. The same properties can be controlled using ZFPs to produce oils with improved characteristics for food and industrial uses.

Detailed Description Text (162):
The primary fatty acids in the TAGs of oilseed crops are 16 to 18 carbons in length and contain 0 to 3 double bonds. Palmitic acid (16:0[16 carbons: 0 double bonds]), oleic acid (18:1), linoleic acid (18:2), and linolenic acid (18:3) predominate. The number of double bonds, or degree of saturation, determines the melting temperature, reactivity, cooking performance, and health attributes of the resulting oil.

Detailed Description Text (163):
The enzyme responsible for the conversion of oleic acid (18:1) into linoleic acid (18:2) (which is then the precursor for 18:3 formation) is .DELTA.12-oleate desaturase, also referred to as omega-6 desaturase. A block at this step in the fatty acid desaturation pathway should result in the accumulation of oleic acid at the expense of polyunsaturates.

Detailed Description Text (164):
In one embodiment ZFPs are used to regulate expression of the FAD2-1 gene in soybeans. Two genes encoding microsomal .DELTA.6 desaturases have been cloned recently from soybean, and are referred to as FAD2-1 and FAD2-2 (Heppard et al., Plant Physiol. 110:311-319 (1996)). FAD2-1 (delta 12 desaturase) appears to control the bulk of oleic acid desaturation in the soybean seed. ZFPs can thus be used to modulate gene expression of FAD2-1 in plants. Specifically, ZFPs can be used to inhibit expression of the FAD2-1 gene in soybean in order to increase the accumulation of oleic acid (18:1) in the oil seed. Moreover, ZFPs can be used to modulate expression of any other plant gene, such as delta-9 desaturase, delta-12 desaturases from other plants, delta-15 desaturase, acetyl-CoA carboxylase, acyl-ACP-thioesterase, ADP-glucose pyrophosphorylase, starch synthase, cellulose synthase, sucrose synthase, senescence-associated genes, heavy metal chelators, fatty acid hydroperoxide lyase, polygalacturonase, EPSP synthase, plant viral genes, plant fungal pathogen genes, and plant bacterial pathogen genes.

Detailed Description Text (173):
Transformed plant cells which are derived by any of the above transformation techniques can be cultured to regenerate a whole plant which possesses the transformed genotype and thus the desired ZFP-controlled phenotype. Such regeneration techniques rely on manipulation of certain phytohormones in a tissue culture growth medium, typically relying on a biocide and/or herbicide marker which has been introduced together with the ZFP nucleotide sequences. Plant regeneration from cultured protoplasts is described in Evans et al., Protoplasts Isolation and Culture, Handbook of Plant Cell Culture, pp. 124-176 (1983); and Binding, Regeneration of Plants, Plant Protoplasts, pp. 21-73 (1985). Regeneration can also be obtained from plant callus, explants, organs, or parts thereof. Such regeneration techniques are described generally in Klee et al. Ann. Rev. of Plant Phys. 38:467-486 (1987).

Detailed Description Text (177):
The ZFP technology can be used to rapidly analyze differential gene expression studies. Engineered ZFPs can be readily used to up or down-regulate any endogenous target gene. Very little sequence information is required to create a gene-specific DNA binding domain. This makes the ZFP technology ideal for analysis of long lists of poorly characterized differentially expressed genes. One can simply build a zinc finger-based DNA binding domain for each candidate gene, create chimeric up and down-regulating artificial transcription factors and test the consequence of up or down-regulation on the phenotype under study (transformation, response to a cytokine etc.) by switching the candidate genes on or off one at a time in a model

system.

Detailed Description Text (185):
The use of engineered ZFPs to manipulate gene expression can be restricted to adult animals using the small molecule regulated systems described in the previous section. Expression and/or function of a zinc finger-based repressor can be switched off during development and switched on at will in the adult animals. This approach relies on the addition of the ZFP expressing module only; homologous recombination is not required. Because the ZFP repressors are trans dominant, there is no concern about germline transmission or homozygosity. These issues dramatically affect the time and labor required to go from a poorly characterized gene candidate (a cDNA or EST clone) to a mouse model. This ability can be used to rapidly identify and/or validate gene targets for therapeutic intervention, generate novel model systems and permit the analysis of complex physiological phenomena (development, hematopoiesis, transformation, neural function etc.). Chimeric targeted mice can be derived according to Hogan et al., Manipulating the Mouse Embryo: A Laboratory Manual, (1988); Teratocarcinomas and Embryonic Stem Cells: A Practical Approach, Robertson, ed., (1987); and Capecchi et al., Science 244:1288 (1989.

Detailed Description Text (192):
This first Example demonstrates the construction of ZFPs designed to recognize DNA sequences contained in the promoter of the human vascular endothelial growth factor (VEGF) gene. VEGF is an approximately 46 kDa glycoprotein that is an endothelial cell-specific mitogen induced by hypoxia. VEGF has been implicated in angiogenesis associated with cancer, various retinopathies, and other serious diseases. The DNA target site chosen was a region surrounding the transcription initiation site of the gene. The two 9 base pair (bp) sites chosen are found within the sequence agcGGGGAGGATcGCGGAGGCTtgg (SEQ ID NO:13), where the upper-case letters represent actual 9-bp targets. The protein targeting the upstream 9-bp target was denoted VEGF1, and the protein targeting the downstream 9-bp target was denoted VEGF3a. The major start site of transcription for VEGF is at the T at the 3' end of the first 9-bp target, which is underlined in the sequence above.

Detailed Description Text (193):
The human SP-1 transcription factor was used as a progenitor molecule for the construction of designed ZFPs. SP-1 has a three finger DNA-binding domain related to the well-studied murine Zif268 (Christy et al., PNAS 85:7857-7861 (1988)). Site-directed mutagenesis experiments using this domain have shown that the proposed "recognition rules" that operate in Zif268 can be used to adapt SP-1 to other target DNA sequences (Desjarlais & Berg, PNAS 91:11099-11103 (1994)). The SP-1 sequence used for construction of zinc finger clones corresponds to amino acids 533 to 624 in the SP-1 transcription factor.

Detailed Description Text (194):
The selection of amino acids in the recognition helices of the two designed ZFPs, VEGF1 and VEGF3a, is summarized in Table 1.

Detailed Description Text (195):
Coding sequences were constructed to express these peptides using a PCR-based assembly procedure that utilizes six overlapping oligonucleotides (FIG. 1). Three oligonucleotides (oligos 1, 3, and 5 in FIG. 1) corresponding to "universal" sequences that encode portions of the DNA-binding domain between the recognition helices. These oligonucleotides remain constant for any zinc finger construct. The other three "specific" oligonucleotides (oligos 2, 4, and 6 in FIG. 1) were designed to encode the recognition helices. These oligonucleotides contained substitutions at positions -1, 2, 3 and 6 on the recognition helices to make them specific for each of the different DNA-binding domains. Codon bias was chosen to allow expression in both mammalian cells and E. coli.

Detailed Description Text (196):
The PCR synthesis was carried out in two steps. First, the double stranded DNA template was created by combining the six oligonucleotides (three universal, three specific) and using a four cycle PCR reaction with a low temperature (25.degree.) annealing step. At this temperature, the six oligonucleotides join to form a DNA "scaffold." The gaps in the scaffold were filled in by a combination of Taq and Pfu polymerases. In the second phase of construction, the zinc finger template was amplified in thirty cycles by external primers that were designed to incorporate restriction sites for cloning into pUC 19. Accuracy of clones for the VEGF ZFPs were verified by DNA sequencing. The DNA sequences of each of the two constructs are listed below. VEGF 1:
GGTACCCATACCTGGCAAGAAGAAGCAGCACATCTGCCACATCCAGGGCTGTGGTAAAGTTTACGGCACAACCT
CAAATCTGCGTCGTCACCTGCGCTGGCACACCGGCGAGAGGCCTTTCATGTGTACCTGGTCCTACTGTGGTAAAC
GCTTCACCCGTTCGTCAAACCTGCAGCGTCACAAGCGTACCCACACCGGTGAGAAGAAATTTGCTTGCCCGGAGT
GTCCGAAGCGCTTCATGCGTAGTGACCACCTGTCCCGTCACATCAAGACCCACCAGAATAAGAAGGGTGGATCC (SEQ ID NO:14) VEGF1 translation:
VPIPGKKKQHICHIQGCGKVYGTTSNLRRHLRWHTGERPFMCTWSYCGKRFTRSSNLQRHKRTHTGEKKFACPE
CPKRFMRSDHLSRHIKTHQNKKGGS (SEQ ID NO:15) VEGF3a:
GGTACCCATACCTGGCAAGAAGAAGCAGCACATCTGCCACATCCAGGGCTGTGGTAAAGTTTACGGCCAGTCCT
CCGACCTGCAGCGTCACCTGCGCTGGCACACCGGCGAGAGGCCTTTCATGTGTACCTGGTCCTACTGTGGTAAAC
GCTTCACCCGTTCGTCAAACCTACAGAGGCACAAGCGTACACACACCGGTGAGAAGAAATTTGCTTGCCCGGAGT
GTCCGAAGCGCTTCATGCGAAGTGACGAGCTGTCACGACATATCAAGACCCACCAGAACAAGAAGGGTGGATCC (SEQ ID NO:16) VEGF3a translation:
VPIPGKKKQHICHIQGCGKVYGQSSDLQRHLRWHTGERPFMCTWSYCGKRFTRSSNLQRHKRTHTGEKKFACPE
CPKRFMRSDELSRHIKTHQNKKGGS (SEQ ID NO:17)

Detailed Description Text (197):
The ability of the designed ZFPs to bind their target sites was verified by expressing and purifying recombinant protein from E. coli and performing electrophoretic mobility shift assays (EMSAs). The expression of ZFPs was carried out in two different systems. In the first, the DNA-binding peptides were expressed in E. coli by inserting them into the commercially available pET15b vector (Novagen). This vector contains a T7 promoter sequence to drive expression of the recombinant protein. The constructs were introduced into E. coli BL21/DE3 (lacI.sup.q) cells, which contain an IPTG-inducible T7 polymerase. Cultures were supplemented with 50 .mu.M ZnCl.sub.2, were grown at 37.degree. C. to an OD at 600 nm of 0.5-0.6, and protein production was induced with IPTG for 2 hrs. ZFP expression was seen at very high levels, approximately 30% of total cellular protein (FIG. 2). These proteins are referred to as "unfused" ZFPs.

Detailed Description Text (198):
Partially pure unfused ZFPs were produced as follows (adapted from Desjarlais & Berg, Proteins: Structure, Function and Genetics 12:101-104 (1992)). A frozen cell pellet was resuspended in 1/50th volume of 1 M NaCl, 25 mM Tris HCl (pH 8.0), 100 .mu.M ZnCl.sub.2, 5 mM DTT. The samples were boiled for 10 min. and centrifuged for 10 min. at .about.3,000.times.g. At this point the ZFP protein in the supernatant was >50% pure as estimated by staining of SDS polyacrylamide gels with Coomassie blue, and the product migrated at the predicted molecular weight of around 11 kDa (FIG. 2).

Detailed Description Text (199):
The second method of producing ZFPs was to express them as fusions to the E. coli Maltose Binding Protein (MBP). N-terminal MBP fusions to the ZFPs were constructed by PCR amplification of the pET15b clones and insertion into the vector pMal-c2 under the control of the Tac promoter (New England Biolabs). The fusion allows simple purification and detection of the recombinant protein. It had been reported previously that zinc finger DNA-binding proteins can be expressed from this vector in soluble form to high levels in E. coli and can bind efficiently to the appropriate DNA target without refolding (Liu et al. PNAS 94:5525-5530 (1997)). Production of MBP-fused proteins was as described by the manufacturer (New England

Biolabs). Transformants were grown in LB medium supplemented with glucose and ampicillin, and were induced with IPTG for 3 hrs at 37.degree. C. The cells were lysed by French press, then exposed to an agarose-based amylose resin, which specifically binds to the MBP moiety, thus acting as an affinity resin for this protein. The MBP fusion protein was eluted with 10 mM maltose (FIG. 2C) to release ZFP of >50% purity. In some cases, the proteins were further concentrated using a Centricon 30 filter unit (Amicon).

Detailed Description Text (200):
Partially purified unfused and MBP fusion ZFPs were tested by EMSA to assess binding to their target DNA sequences. The protein concentrations in the preparations were measured by Bradford assay (BioRad). Since SDS polyacrylamide gels demonstrated >50% homogeneity by either purification method, no adjustment was made for ZFP purity in the calculations. In addition, there could be significant amounts of inactive protein in the preparations. Therefore, the data generated by EMSAs below represent an underestimate of the true affinity of the proteins for their targets (i.e., overestimate of K.sub.d s). Two separate preparations were made for each protein to help control for differences in ZFP activity.

Detailed Description Text (201):
The VEGF DNA target sites for the EMSA experiments were generated by embedding the 9-bp binding sites in 29-bp duplex oligonucleotides. The sequences of the recognition ("top") strand and their complements ("bottom") used in the assays are as follows: VEGF site 1, top: 5'-CATGCATAGCGGGGAGGATCGCCATCGAT (SEQ ID NO:18) VEGF site 1, bottom: 5'-ATCGATGGCGATCCTCCCCGCTATGCATG (SEQ ID NO:19) VEGF site 3, top: 5'-CATGCATATCGCGGAGGCTTGGCATCGAT (SEQ ID NO:20) VEGF site 3, bottom: 5'-ATCGATGCCAAGCCTCCGCGATATGCATG (SEQ ID NO:21)

Detailed Description Text (202):
The VEGF DNA target sites are underlined. The 3 bp on either side of the 9 bp binding site was also derived from the actual VEGF DNA sequence. The top strand of each target site was labeled with polynucleotide kinase and .gamma.-.sup.32 P dATP. Top and bottom strands were annealed in a reaction containing each oligonucleotide at 0.5 .mu.M, 10 mM Tris-HCl (pH 8.0), 1 mM EDTA, and 50 mM NaCl. The mix was heated to 95.degree. C. for 5 min. and slow cooled to 30.degree. C. over 60 min. Duplex formation was confirmed by polyacrylamide gel electrophoresis. Free label and ssDNA remaining in the target preparations did not appear to interfere with the binding reactions.

Detailed Description Text (203):
Binding of the ZFPs to target oligonucleotides was performed by titrating protein against a fixed amount of duplex substrate. Twenty microliter binding reactions contained 10 ftnole (0.5 nM) 5'-.sup.32 P-labeled double-stranded target DNA, 35 mM Tris HCl (pH 7.8), 100 mM KCl, 1 mM MgCl.sub.2, 1 mM dithiothreitol, 10% glycerol, 20 .mu.g/ml poly dI-dC (optionally), 200 .mu.g/ml bovine serum albumin, and 25 .mu.M ZnCl.sub.2. Protein was added as one fifth volume from a dilution series made in 200 mM NaCl, 20 mM Tris (pH 7.5), 1 mM DTT. Binding was allowed to proceed for 30 min. at room temperature. Polyacrylamide gel electrophoresis was carried out at 4.degree. C. using precast 10% or 10-20% Tris-HCl gels (BioRad) and standard Tris-Glycine running buffer containing 0.1 mM ZnCl.sub.2.

Detailed Description Text (204):
The results of a typical EMSA using an MBP fused ZFP are shown in FIG. 3. In this case, a 3-fold dilution series of the MBP-VEGF1 protein was used. The shifted product was quantitated on a phosphorimager (Molecular Dynamics) and the relative signal (percent of plateau value) vs. the log.sub.10 of nM protein concentration was plotted. An apparent K.sub.d was found by determining the protein concentration that gave half maximal binding of MBP-VEGF1 to its target site, which in this experiment was approximately 2 nM.

Detailed Description Text (205):
The binding affinities determined for the VEGF proteins can be summarized as follows. VEGF1 showed the stronger DNA-binding affinity; in multiple EMSA analyses, the average apparent K.sub.d was determined to be approximately 10 nM when bound to VEGF site 1. VEGF3a bound well to its target site but with a higher apparent K.sub.d than VEGF1; the average K.sub.d for VEGF3a was about 200 nM. In both cases the MBP-fused and unfused versions of the proteins bound with similar affinities. K.sub.d s were also determined under these conditions for MBP fusions of the wild-type Zif268 and SP-1 ZFPs, which yielded K.sub.d s of 60 and 65 nM, respectively. These results are similar to binding constants reported in the literature for Zif268 of approximately 2-30 nM (see, e.g., Jamieson et al., Biochemistry 33:5689-5695 (1994)). The K.sub.d s for the synthetic VEGF ZFPs therefore compare very favorably with those determined for these naturally-occurring DNA-binding proteins.

Detailed Description Text (206):
In summary, this Example demonstrates the generation of two novel DNA-binding proteins directed to specific targets near the transcriptional start of the VEGF gene. These proteins bind with affinities similar to those of naturally-occurring transcription factors binding to their targets.

Detailed Description Text (209):
An important consideration in ZFP design is DNA target length. For random DNA, a sequence of n nucleotides would be expected to occur once every 0.5.times.4.sup.n base-pairs. Thus, DNA-binding domains designed to recognize only 9 bp of DNA would find sites every 130,000 bp and could therefore bind to multiple locations in a complex genome (on the order of 20,000 sites in the human genome). 9-bp putative repressor-binding sequences have been chosen for VEGF in the 5' UTR where they might directly interfere with transcription. However, in case zinc finger domains that recognize 9-bp sites lack the necessary affinity or specificity when expressed inside cells, a larger domain was constructed to recognize 18 base-pairs by joining separate three-finger domains with a linker sequence to form a six-finger protein. This should ensure that the repressor specifically targets the appropriate sequence, particularly under conditions where only small amounts of the repressor are being produced. The 9-bp target sites in VEGF were chosen to be adjacent to one another so that the zinc fingers could be linked to recognize an 18-bp sequence. The linker DGGGS (SEQ ID NO:4) was chosen because it permits binding of ZFPs to two 9-bp sites that are separated by a one nucleotide gap, as is the case for the VEGF1 and VEGF3a sites (see also Liu et al., PNAS 5525-5530 (1997)).

Detailed Description Text (210):
The 6-finger VEGF3a/1 protein encoding sequence was generated as follows. VEGF3a was PCR amplified using the primers SPE7 (5'-GAGCAGAATTCGGCAAGAAGAAGCAGCAC (SEQ ID NO:22)) and SPEamp12 (5'-GTGGTCTAGACAGCTCGTCACTTCGC (SEQ ID NO:23)) to generate EcoRI and XbaI restriction sites at the ends (restriction sites underlined). VEGF1 was PCR amplified using the primers SPEamp13 (5'-GGAGCCAAGGCTGTGGTAAAGTTTACGG (SEQ ID NO:24)) and SPEamp11 (5'-GGAGAAGCTTGGATCCTCATTATCCC (SEQ ID NO:25)) to generate StyI and HindIII restriction sites at the ends (restriction sites underlined). Using synthetic oligonucleotides, the following sequence was ligated between the XbaI and StyI sites, where XbaI and StyI are underlined: TCT AGACAC ATC AAA ACC CAC CAG AAC AAG AAA GAC GGC GGT GGC AGC GGC AAA AAG AAA CAG CAC ATA TGT CAC ATC CAA GG (SEQ ID NO:26). This introduced the linker sequence DGGGS (SEQ ID NO:4) between the two SP-1 domains. The ligation product was reamnplified with primers SPE7 and SPEamp11 and cloned into pUC19 using the EcoRI and HindIII sites. The linked ZFP sequences were then amplified with primers (1) GB19 GCCATGCCGGTACCCATACCTGGCAAGAAGAAGCAGCAC (SEQ ID NO:27) (2) GB10 CAGATCGGATCCACCCTTCTTATTCTGGTGGGT (SEQ ID NO:28) to introduce KpnI and BamHI sites for cloning into the modified pMAL-c2 expression vector as described above.

Detailed Description Text (211):

h e b b g e e e f c e b e ge

The nucleotide sequence of the designed, 6-finger ZFP VEGF3a/1 from KpnI to BamHI is: GGTACCCATACCTGGCAAGAAGAAGCAGCACATCTGCCACATCCAGGGCTGTGGTAAAGTTTACGGCCAGTCCT
CCGACCTGCAGCGTCACCTGCGCTGGCACACCGGCGAGAGGCCTTTCATGTGTACCTGGTCCTACTGTGGTAAAC
GCTTCACACGTTCGTCAAACCTACAGAGGCACAAGCGTACACACACAGGTGAGAAGAAATTTGCTTGCCCGGAGT
GTCCGAAGCGCTTCATGCGAAGTGACGAGCTGTCTAGACACATCAAAACCCACCAGAACAAGAAAGACGGCGGTG
GCAGCGGCAAAAAGAAACAGCACATATGTCACATCCAAGGCTGTGGTAAAGTTTACGGCACAACCTCAAATCTGC
GTCGTCACCTGCGCTGGCACACCGGCGAGAGGCCTTTCATGTGTACCTGGTCCTACTGTGGTAAACGCTTCACCC
GTTCGTCAAACCTGCAGCGTCACAAGCGTACCCACACCGGTGAGAAGAAATTTGCTTGCCCGGAGTGTCCGAAGC
GCTTCATGCGTAGTGACCACCTGTCCCGTCACATCAAGACCCACCAGAATAAGAAGGGTGGATCC (SEQ ID NO:29)

Detailed Description Text (212):
The VEGF3a/1 amino acid translation (using single letter code) is:
VPIPGKKKQHICHIQGCGKVYGQS
SDLQRHLRWHTGERPFMCTWSYCGKRFTRSSNLQRHKRTHTGEKKFACPECPKRFMRSDELSRHIKTHQNKKDG
GGSGKKKQHICHIQGCGKVYGTTSNLRRHLRWHTGERPFMCTWSYCGKRFTRSSNLQRHKRTHTGEKKFACPECP
KRFMRSDHLSRHIKTHQNKKGGS (SEQ ID NO:30)

Detailed Description Text (213):
The 18-bp binding protein VEGF3a/1 was expressed in E. coli as an MBP fusion, purified by affinity chromatography, and tested in EMSA experiments as described in Example 1. The target oligonucleotides were prepared as described and comprised the following complementary sequences: (1) JVF9
AGCGAGCGGGGAGGATCGCGGAGGCTTGGGGCAGCCGGGTAG (SEQ ID NO:31), and (2) JVF10
CGCTCTACCCGGCTGCCCCAAGCCTCCGCGATCCTCCCCGCT (SEQ ID NO:32).

Detailed Description Text (214):
For the EMSA studies, 20 .mu.l binding reactions contained 10 fmole (0.5 nM) 5'-.sup.32 P-labeled double-stranded target DNA, 35 mM Tris HCl (pH 7.8), 100 mM KCl, 1 mM MgCl.sub.2, 5 mM dithiothreitol, 10% glycerol, 20 .mu.g/ml poly dI-dC, 200 .mu.g/ml bovine serum albumin, and 25 .mu.M ZnCl.sub.2. Protein was added as one fifth volume from a 3-fold dilution series. Binding was allowed to proceed for 60 min at either room temperature or 37.degree. C. Polyacrylamide gel electrophoresis was carried out at room temperature or 37.degree. C. using precast 10% or 10-20% Tris-HCl gels (BioRad) and standard Tris-Glycine running buffer. The room temperature assays yielded an apparent K.sub.d for this VEGF3a/1 protein of approximately 1.5 nM. Thus, the 18-bp binding ZFP bound with high affinity to its target site. In a parallel experiment, VEGF1 protein was tested against its target using the oligonucleotides described in Example I, yielding an apparent K.sub.d of approximately 2.5 nM. When binding and electrophoresis were performed at 37.degree. C., the apparent K.sub.d of VEGF3a/1 was approximately 9 nM when tested against the 18-bp target, compared to a K.sub.d of 40 nM for VEGF1 tested against its target. This indicates that the difference in binding affinities is accentuated at the higher temperature.

Detailed Description Text (215):
The apparent K.sub.d is a useful measure of the affinity of a protein for its DNA target. However, for a DNA binding site either in vitro or in vivo, its occupancy is determined to a large extent by the off-rate of the DNA-binding protein. This parameter can be measured by competition experiments as shown in FIG. 4. The conditions for EMSA were as described above; binding and electrophoresis were performed at 37.degree. C. These data indicate that the half-life of the protein-DNA complex is more than ten times longer for VEGF3a/1 than for VEGF1. Thus, under these in vitro conditions, the occupancy of the target site is much higher for the 18-bp binding protein than for the 9-bp binding protein.

Detailed Description Text (219):
Certain DNA-binding proteins contain separable domains that function as transcriptional repressors. Approximately 20% of ZFPs contain a non-DNA-binding domain of about 90 amino acids that functions as a transcriptional repressor (Thiesen, The New Biologist 2:363-374 (1990); Margolin et al., PNAS 91:4509-4513

(1994); Pengue et al., (1994), supra; Witzgall et al., (1994), supra). This domain, termed the KRAB domain, is modular and can be joined to other DNA-binding proteins to block expression of genes containing the target DNA sequence (Margolin et al., (1994); Pengue et al., (1994); Witzgall et al., (1994), supra). The KRAB domain has no effect by itself; it needs to be tethered to a DNA sequence via a DNA-binding protein to function as a repressor. The KRAB domain has been shown to block transcription initiation and can function at a distance of up to at least 3 kb from the transcription start site. The KRAB domain from the human KOX-1 protein (Thiesen, The New Biologist 2:363-37 (1990)) was used for the studies described here. This 64 amino acid domain can be fused to ZFPs and has been shown to confer repression in cell culture (Liu et al., supra).

Detailed Description Text (220):
The VP16 protein of HSV-1 has been studied extensively, and it has been shown that the C-terminal 78 amino acids can act as a trans-activation domain when fused to a DNA-binding domain (Hagmann et al., J. Virology 71:5952-5962 (1997)). VP16 has also been shown to function at a distance and in an orientation-independent manner. For these studies, amino acids 413 to 490 in the VP16 protein sequence were used. DNA encoding this domain was PCR amplified from plasmid pMSVP16.DELTA.C+119 using primers with the following sequences: (1) JVF24 CGCGGATCCGCCCCCCCGACCGATG (SEQ ID NO:33), and (2) JVF25
CCGCAAGCTTACTTGTCATCGTCGTCCTTGTAGTCGCTGCCCCCACCGTACTCGTCAATTCC (SEQ ID NO:34).

Detailed Description Text (223):
The vectors were constructed as follows. Plasmid pcDNA-.DELTA.HB was constructed by digesting plasmid pcDNA-3.1(+) (Invitrogen) with HindIII and BamHI, filling in the sticky ends with Klenow, and religating. This eliminated the HindIII, KpnI, and BamHI sites in the polylinker. The vector pcDNA3.1(+) is described in the Invitrogen catalog. Plasmid pcDNA-NKF was generated by inserting a fragment into the EcoRI/XhoI sites of pcDNA-.DELTA.HB that contained the following: 1) a segment from EcoRI to KpnI containing the Kozak sequence including the initiation codon and the SV40 NLS sequence, altogether comprising the DNA sequence
GAATTCGCTAGCGCCACCATGGCCCCCAAGAAGAAGAGGAAGGTGGGAATCCATGGGGTAC(SEQ ID NO:36), where the EcoRI and KpnI sites are underlined; and 2) a segment from KpnI to XhoI containing a BamHI site, the KRAB-A box from KOX1 (amino acid coordinates 11-53 in Thiesen, 1990, supra), the FLAG epitope (from Kodak/IBI catalog), and a HindIII site, altogether comprising the sequence
GGTACCCGGGGATCCCGGACACTGGTGACCTTCAAGGATGTATTTGTGGACTTCACCAGGGAGGAGTGGAAGCT
GCTGGACACTGCTCAGCAGATCGTGTACAGAAATGTGATGCTGGAGAACTATAAGAACCTGGTTTCCTTGGGCAG
CGACTACAAGGACGACGATGACAAGTAAGCTTCTCGAG (SEQ ID NO:37) where the KpnI, BamHI and XhoI sites are underlined.

Detailed Description Text (229):
Finally, control versions of both the KRAB and VP16 expression plasmids were constructed. Plasmid NKF-control was designed to express NLS-KRAB-FLAG without zinc finger protein sequences; plasmid NVF-control was designed to express NLS-VP16-FLAG without ZFP sequences. These plasmids were made by digesting pcDNA-NKF and -NVF, respectively, with BamHI, filling in the ends with Klenow, and religating in order to place the downstream domains into the proper reading frame. These plasmids serve as rigorous controls for cell culture studies.

Detailed Description Text (233):
This Example demonstrates the use of transient co-transfection studies to measure the activity of the ZFP repressor proteins in cells. Such experiments involve co-transfection of ZFP-KRAB expression ("effector") plasmids with reporter plasmids carrying the VEGF target sites. Efficacy is assessed by the repression of reporter gene expression in the presence of the effector plasmid relative to empty vector controls.

Detailed Description Text (235):

The first six and last six nucleotides shown are the MluI and BglII sites; the lowercase letters indicate HindIII sites. The binding sites for VEGF1 and VEGF3a are underlined.

Detailed Description Text (238):
For the control reporter plasmid pGL3-Control (pGL3-C), the presence or absence of the ZFP-KRAB expression plasmid does not influence the luciferase expression level. However, for pVFR1-4x, the reporter containing four copies of the VEGF target site, presence of the VEGF1 (9-bp-binding ZFP) or VEGF3a/1 (18-bp-binding ZFP) expression plasmid reduces luciferase expression by a factor of 2-3 relative to the empty pcDNA vector control. The VEGF3a (9-bp-binding ZFP) expression plasmid appears to exhibit little or no effect. These experiments clearly demonstrate that a designed ZFP is capable of functioning in a cell to repress transcription of a gene when its target site is present. Furthermore, it appears that a certain level of affinity is required for function; i.e., VEGF1 and VEGF3a/1, with K.sub.d s of 10 nM or less, are functional, whereas VEGF3a, with a K.sub.d of 200 nM, is not.

Detailed Description Text (246):
For the control reporter plasmid, pGL3-Promoter (pGL3-P), the presence or absence of the ZFP-VP16 expression plasmid does not significantly influence the luciferase expression level. For pVFR3-4x, the reporter containing four copies of the VEGF target site, presence of VEGF1 (the 9-bp-binding ZFP) shows a very slight activation relative to the empty pcDNA vector control. VEGF3a/1 (the 18-bp-binding ZFP) expression plasmid activates luciferase expression very substantially, showing about a 14-fold increase relative to pcDNA. These experiments clearly demonstrate that a designed ZFP, when fused to the VP16 activation domain, is capable of functioning in a cell to activate transcription of a gene when its target site is present. Furthermore, these results clearly demonstrate that an 18-bp binding protein, VEGF3a/1, is a much better activator in this assay than a 9-bp binding VEGF1 protein. This could be a result of the improved affinity or decreased off-rate of the VEGF3a/1 protein.

Detailed Description Text (255):
A 40-50-fold decrease in VEGF expression was noted in the DFX treated cells transfected with VEGF3a/1-KRAB, an expression vector encoding the 18 bp binding VEGF high affinity ZFP. A two-fold decrease in expression was observed when cells were transfected with VEGF1-KRAB, an expression vector encoding the 9 bp binding VEGF high affinity ZFP. No significant decrease in VEGF expression was observed in cells that were transfected with a non-VEGF ZFP (CCR5-KRAB) or NKF-control (FIG. 8). Similar results have been obtained in three independent transfection experiments.

Detailed Description Text (256):
In a separate experiment, the following results were obtained (data not shown). VEGF1-NF, which expresses the 9-bp-binding VEGF1 ZFP without a functional domain, showed no effect on VEGF gene expression. A significant reduction in VEGF expression was observed with VEGF3a/1-NF, which expresses the 18-bp binding protein without a functional domain. This result suggests that binding to the start site of transcription, even without a repression domain, interferes with transcription. Even when fused to the KRAB domain, the VEGF3a ZFP is unable to affect expression levels (plasmid VEGF3a-KRAB). However, VEGF1 fused to KRAB (VEGF1-KRAB) results in a dramatic decrease in expression. VEGF3a/1 fused to KRAB (VEGF3a/1-KRAB) prevents expression of VEGF altogether.

Detailed Description Text (257):
These data indicate that a designed ZFP is capable of locating and binding to its target site on the chromosome and preventing expression of an endogenous cellular target gene. In particular, the results indicate that ZFPs with a K.sub.d of less than about 25 nM (e.g., VEGF1 has an average apparent K.sub.d of about 10 nM) provide dramatic decreases in expression. In addition, the data demonstrate that

the KRAB functional domain enhances gene silencing. Because in this experiment the introduction of the repressor occurs before the inducer of VEGF is added (DFX), the data demonstrate the ability of a designed repressor to prevent activation of an already quiescent gene. In addition, these results demonstrate that a six-finger engineered ZFP (VEGF3a/1) with nanomolar affinity for its target is able to inhibit the hypoxic response of the VEGF gene when it binds a target that overlaps the transcriptional start site.

Detailed Description Text (262):
For the three-fingered VEGF1-specific ZFP (VEGF1-VP16), a 7-10 fold increase in VEGF expression was observed when compared to control plasmid (NVF-control) and mock transfected cells (FIG. 9). Similar results have been obtained in 5 independent experiments. It is important to note that the level of VEGF secretion in VEGF1-VP16 transfected cells was equivalent or greater than the level in cells that have been treated with DFX (FIG. 9). Introduction of VEGF3a/1-VP16 stimulated a more modest induction of VEGF. This result is consistent with the finding in Example VI, in which expression of the 18-bp binding protein without a functional domain prevented activation to a certain degree. This result suggested that the tight binding of this protein to the start site of transcription interferes with activation.

Detailed Description Text (263):
These data indicate that a designed ZFP is capable of locating and binding to its target site on the chromosome, presenting a transcriptional activation domain, and dramatically enhancing the expression level of that gene. In particular, the results indicate that ZFPs with a K.sub.d of less than about 25 nM (e.g., VEGF1 has an average apparent K.sub.d of about 10 nM) provide dramatic increases in expression.

Detailed Description Text (266):
To further substantiate the results in Examples VI and VII, a ribonuclease protection assay (RPA) was performed to correlate the increased level of VEGF protein with an increase in VEGF mRNA levels (Example VII), and to correlate the decreased level of VEGF protein with a decrease in VEGF mRNA levels (Example VI).

Detailed Description Text (269):
This experiment demonstrates that the increase in VEGF protein observed upon transfection with the VEGF1-VP16 chimeric transcription factor is mediated by an increase in the level of VEGF mRNA. Similarly, the decrease in VEGF protein observed upon transfection with the VEGF3a/1-KRAB chimeric transcription factor is mediated by a decrease in the level of VEGF mRNA.

Detailed Description Text (273):
The EPO2C ZFP was designed to recognize a 9-bp DNA-binding site located 853-bp upstream of the EPO transcription initiation site. Methods for design and construction of EPO2C are described herein and in U.S. Pat. No. 6,453,242, filed Jan. 12, 1999. The EPO2C binding site sequence is GCGGTGGCT.

Detailed Description Text (275):
2.times.10.sup.6 Hep3B cells (a human hepatocellular carcinoma-derived cell line) or 5.times.10.sup.6 HEK293 cells (a human embryonic kidney epithelium-derived cell line) were seeded into 6-well plates one day before transfection. 500 ng of the effector plasmid (encoding the engineered ZFP) was transiently transfected into the cells using Lipofectamin (GIBCO-BRL). Mock transfection and transfection with an empty expression vector served as controls. One day later the growth medium was removed, and fresh DMEM was added. Culture supernatants were collected 24 hours later for determination of EPO protein expression levels using a commercially available ELISA kit (R&D Systems).

Detailed Description Text (276):

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The results in FIG. 11a show that transfection of a vector encoding the EPO2C ZFP transactivation protein significantly increased the level of EPO expression when compared to control vector (pcDNANVF) or mock transfected cells. This activation was observed in both Hep3B and HEK293 cells (FIG. 11a).

Detailed Description Paragraph Equation (4):
[protein]=(25.times.10.sup.-9 moles/L) (10.sup.-12 L/nucleus) (6.times.10.sup.23 molecules/mole)=15,000 molecules/nucleus for 50% binding

Detailed Description Paragraph Equation (6):
(2.5.times.10.sup.-6 moles/L) (10.sup.-12 L/nucleus) (6.times.10.sup.-23 molecules/mole)=about 1,500,000 molecules per nucleus for 99% binding of target site.

Detailed Description Paragraph Table (1):
TABLE 1 Amino acids chosen for recognition helices of VEGF-recognizing ZFPs Position: Finger 1 Finger 2 Finger 3 Protein -1 2 3 6 -1 2 3 6 -1 2 3 6 VEGF1 T S N R R S N R R D H R VEGF3A Q S D R R S N R R D E R

Detailed Description Paragraph Table (2):
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Sequencelinker <400> SEQUENCE: 12 Leu Arg Gln Lys Asp Gly Gly Gly Ser Gly Gly Gly Ser Glu Arg Pro 1 5 10 15 <200> SEQUENCE CHARACTERISTICS: <210> SEQ ID NO 13 <211> LENGTH: 25 <212> TYPE: DNA <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Description of Artificial SequenceZFP target site region surrounding initiation site of vascular endothelial growth factor (VEGF) gene containing two 9-base pair target sites <400> SEQUENCE: 13 agcggggagg atcgcggagg cttgg 25 <200> SEQUENCE CHARACTERISTICS: <210> SEQ ID NO 14 <211> LENGTH: 298 <212> TYPE: DNA <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Description of Artificial SequenceVEGF1 ZFP construct targeting upstream 9-base pair target site in VEGF promoter <400> SEQUENCE: 14 g gta ccc ata cct ggc aag aag aag cag cac atc tgc cac atc cag ggc 49 Val Pro Ile Pro Gly Lys Lys Lys Gln His Ile Cys His Ile Gln Gly 1 5 10 15 tgt ggt aaa gtt tac ggc aca acc tca aat ctg cgt cgt cac ctg cgc 97 Cys Gly Lys Val Tyr Gly Thr Thr Ser Asn Leu Arg Arg His Leu Arg 20 25 30 tgg cac acc ggc gag agg cct ttc atg tgt acc tgg tcc tac tgt ggt 145 Trp His Thr Gly Glu Arg Pro Phe Met Cys Thr Trp Ser Tyr Cys Gly 35 40 45 aaa cgc ttc acc cgt tcg tca aac ctg cag cgt cac aag cgt acc cac 193 Lys Arg Phe Thr Arg Ser Ser Asn Leu Gln Arg His Lys Arg Thr His 50 55 60 acc ggt gag aag aaa ttt gct tgc ccg gag tgt ccg aag cgc ttc atg 241 Thr Gly Glu Lys Lys Phe Ala Cys Pro Glu Cys Pro Lys Arg Phe Met 65 70 75 80 cgt agt gac cac ctg tcc cgt cac atc aag acc cac cag aat aag aag 289 Arg Ser Asp His Leu Ser Arg His Ile Lys Thr His Gln Asn Lys Lys 85 90 95 ggt gga tcc 298 Gly Gly Ser <200> SEQUENCE CHARACTERISTICS: <210> SEQ ID NO 15 <211> LENGTH: 99 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Description of Artificial SequenceVEGF1 ZFP construct targeting upstream 9-base pair target site in VEGF promoter <400> SEQUENCE: 15 Val Pro Ile Pro Gly Lys Lys Lys Gln His Ile Cys His Ile Gln Gly 1 5 10 15 Cys Gly Lys Val Tyr Gly Thr Thr Ser Asn Leu Arg Arg His Leu Arg 20 25 30 Trp His Thr Gly Glu Arg Pro Phe Met Cys Thr Trp Ser Tyr Cys Gly 35 40 45 Lys Arg Phe Thr Arg Ser Ser Asn Leu Gln Arg His Lys Arg Thr His 50 55 60 Thr Gly Glu Lys Lys Phe Ala Cys Pro Glu Cys Pro Lys Arg Phe Met 65 70 75 80 Arg Ser Asp His Leu Ser Arg His Ile Lys Thr His Gln Asn Lys Lys 85 90 95 Gly Gly Ser <200> SEQUENCE CHARACTERISTICS: <210> SEQ ID NO 16 <211> LENGTH: 298 <212> TYPE: DNA <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Description of Artificial SequenceVEGF3a ZFP construct targeting downstream 9-base pair target site in VEGF promoter <400> SEQUENCE: 16 g gta ccc ata cct ggc aag aag aag cag cac atc tgc cac atc cag ggc 49 Val Pro Ile Pro Gly Lys Lys Lys Gln His Ile Cys His Ile Gln Gly 1 5 10 15 tgt ggt aaa gtt tac ggc cag tcc tcc gac ctg cag cgt cac ctg cgc 97 Cys Gly Lys Val Tyr Gly Gln Ser Ser Asp Leu Gln Arg His Leu Arg 20 25 30 tgg cac acc ggc gag agg cct ttc atg tgt acc tgg tcc tac tgt ggt 145 Trp His Thr Gly Glu Arg Pro Phe Met Cys Thr Trp Ser Tyr Cys Gly 35 40 45 aaa cgc ttc acc cgt tcg tca aac cta cag agg cac aag cgt aca cac 193 Lys Arg Phe Thr Arg Ser Ser Asn Leu Gln Arg His Lys Arg Thr His 50 55 60 acc ggt gag aag aaa ttt gct tgc ccg gag tgt ccg aag cgc ttc atg 241 Thr Gly Glu Lys Lys Phe Ala Cys Pro Glu Cys Pro Lys Arg Phe Met 65 70 75 80 cga agt gac gag ctg tca cga cat atc aag acc cac cag aac aag aag 289 Arg Ser Asp Glu Leu Ser Arg His Ile Lys Thr His Gln Asn Lys Lys 85 90 95 ggt gga tcc 298 Gly Gly Ser <200> SEQUENCE CHARACTERISTICS: <210> SEQ ID NO 17 <211> LENGTH: 99 <212> TYPE: PRT <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Description of Artificial SequenceVEGF3a ZFP construct targeting downstream 9-base pair target site in VEGF promoter <400> SEQUENCE: 17 Val Pro Ile Pro Gly Lys Lys Lys Gln His Ile Cys His Ile Gln Gly 1 5 10 15 Cys Gly Lys Val Tyr Gly Gln Ser Ser Asp Leu Gln Arg His Leu Arg 20 25 30 Trp His Thr Gly Glu Arg Pro Phe Met Cys Thr Trp Ser Tyr Cys Gly 35 40 45 Lys Arg Phe Thr Arg Ser Ser Asn Leu Gln Arg His Lys Arg Thr His 50 55 60 Thr Gly Glu Lys Lys Phe Ala Cys Pro Glu Cys Pro Lys Arg Phe Met 65 70 75 80 Arg Ser Asp Glu Leu Ser Arg His Ile Lys Thr His Gln Asn Lys Lys 85 90 95 Gly Gly Ser <200> SEQUENCE CHARACTERISTICS: <210> SEQ ID NO 18 <211> LENGTH: 29 <212> TYPE: DNA <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Description of Artificial SequenceVEGF DNA target site 1 recognition (top) strand

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Current US Original Classification (1):
435/6

Other Reference Publication (1):
Gumucio et al, Interaction of Sp1 With the Human Globin Promoter: Binding and Transactivation of Normal and Mutant Promoters, Oct. 1991, Blood, vol. 78, No. 7, pp. 1853-1863.*

Other Reference Publication (3):
Liu et al, Design of polydactyl zinc-finger proteins for unique addressing within complex genomes, May 1997, Biochemistry, vol. 94, pp. 5525-5530.

Other Reference Publication (4):
Jeremy M. Berg, Sp1 and the subfamily of zinc finger proteins with guarine-rich, Dec. 1992, Commentary, Berg, vol. 89. pp 11109-11110.

Other Reference Publication (8):
Wu et al, Human Immunodeficiency Virus Type 1 Nucleocapsid Protein Reduces Reverse Transcriptase Pausing at a Secondary Structure near the Murine Leukemia Virus Polypurine Tract, Oct. 1996, Journal of Virology, vol. 70 pp. 7132-7142.*

Other Reference Publication (11):

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Bonde et al., "Ontogeny of the v-erbA Oncoprotein from the Thyroid Hormone Receptor: An Alteration in the DNA Binding Domain Plays a Role Crucial for verbA Function," J. Virology 65(4):2037-2046 (1991).

Other Reference Publication (12):
DesJardins et al., "Repeated CT Elements Bound by Zinc Finger Proteins Control the Absolute and Relative Activities of the Two Principal Human C-myc Promoters," Mol. Cell. Biol. 13(9):5710-5724 (1993).

Other Reference Publication (13):
Ghosh "A relational database of transcription factors," Nucleic Acids Res 18:1749-1756 (1990).

Other Reference Publication (14):
Hall et al., "Functional Interaction Between the Two Zinc Finger Domains of the V-erbA Oncoprotein," Cell Growth & Differentiation 3:207-216 (1992).

Other Reference Publication (17):
DesJarlais et al., "Toward rules relating zinc finger protein sequences and DNA binding site preferences," Proc. Natl. Acad. Sci, (1992), 89:7345-7349, USA.

Other Reference Publication (18):
Imhof et al., "Transcriptional Regulation of the AP-2alpha Promoter by BTEB-1 and AP-2REP, a Novel WT-1/EGR-Related Zinc Finger Repressor," Molecular and Cellular Biology 19(1):194-204 (1999).

Other Reference Publication (19):
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CLAIMS:

1. A method of activating expression of a developmentally silenced endogenous cellular gene in a cell; the method comprising the steps of: (a) administering a nucleic acid to the cell, wherein the nucleic acid comprises a sequence encoding a first zinc finger protein operably linked to a promoter, wherein the first zinc finger protein is engineered; and (b) contacting a first target site in the endogenous cellular gene with the first zinc finger protein, wherein the Kd of the zinc finger protein is less than about 25 nM; wherein said contacting results in activation of expression of the endogenous cellular gene to at least about 150%.

3. The method of claim 1, wherein the step of contacting further comprises contacting a second target site in the endogenous cellular gene with a second zinc finger protein.

5. The method of claim 4, wherein the first and second zinc finger proteins are covalently linked.

6. The method of claim 3, wherein the first and second zinc finger proteins are fusion proteins, each comprising a regulatory domain.

7. The method of claim 6, wherein the first and the second zinc finger protein are fusion proteins, each comprising at least two regulatory domains.

8. The method of claim 1, wherein the first zinc finger protein is a fusion protein comprising a regulatory domain.

9. The method of claim 8, wherein the first zinc finger protein is a fusion protein comprising at least two regulatory domains.

16. The method of claim 1, wherein the nucleic acid is administered to the cell in a lipid:nucleic acid complex or as naked nucleic acid.

17. The method of claim 1, wherein the first zinc finger protein is encoded by an expression vector comprising a zinc finger protein nucleic acid operably linked to a promoter, and wherein the method further comprises the step of first administering the expression vector to the cell.

20. The method of claim 17, wherein the first zinc finger protein is encoded by a nucleic acid operably linked to an inducible promoter.

21. The method of claim 17, wherein the first zinc finger protein is encoded by a nucleic acid operably linked to a weak promoter.

22. The method of claim 1, wherein the cell comprises less than about 1.5.times.10.sup.6 copies of the first zinc finger protein.

26. The method of claim 1, wherein the first zinc finger protein comprises an SP-1 backbone.

27. The method of claim 26, wherein the first zinc finger protein comprises a regulatory domain and is humanized.

32. A method of activating expression of a developmentally silenced endogenous cellular gene in a cell the method comprising the steps of: (a) administering a nucleic acid to the cell, wherein the nucleic acid comprises a sequence encoding a fusion zinc finger protein operably linked to a promoter, wherein the fusion zinc finger protein comprises six fingers and a regulatory domain; and (b) contacting a first target site in the endogenous cellular gene with the fusion zinc finger protein, wherein the K.sub.d of the zinc finger protein is less than about 25 nM; thereby activating expression of the endogenous cellular gene to at least about 150%.